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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

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Effectiveness of the Integument and Gastric Fluid as Barriers Against Transmission of *Gaffkya homari* to the Lobster *Homarus americanus*

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A rupture of the integument was shown to be essential for the transmission of *Gaffkya homari* to the lobster *Homarus americanus.* The acidity of the lobster gastric fluid was bactericidal for *G. homari* and was an adequate barrier against transmission of gaffkemia by the oral route. Lytic activity associated with the larger protein molecules of lobster gastric fluid, but not with the bactericidal effect of the acid condition, was weakly active against *Micrococcus lysodeikticus* but was not active against *G. homari.* The acidity of the lobster stomach is a function of the acidic protein molecules rather than a result of an excess of small, acidic molecules.

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INTRODUCTION

THE SEVERITY OF GAFFKEMIA, the fatal infection of lobsters by *Gaffkya homari* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947; and Roskam, 1957), stems from the lobster's complete lack of intrinsic defense mechanisms against a virulent strain of the microorganism (Cornick and Stewart, 1968a). Despite this, the epizootics of this disease which occur periodically in large groups of lobsters held live in commercial operations do not result in the deaths of all of the captive animals (Snieszko and Taylor, 1947; Stewart et al., 1966).

The introduction of the pathogen into commercial holding units is a constant hazard since the pathogen can be present in apparently healthy lobsters at the time of capture (Stewart and MacDonald, 1962; Rabin, 1965; Wood, 1965a; Stewart et al., 1966). The chances for contact with the pathogen, when it is present under the crowded commercial conditions, are excellent. The lobsters' cannibalistic instincts almost certainly ensure that weak or dead lobsters, including those suffering from gaffkemia, will be eaten. In the course of eating infected lobsters a lobster would be expected to consume a dose of *G. homari* larger than the minimum shown by Cornick and Stewart (1968a) to cause a fatal infection when injected. Other lobsters in the general area but not involved in a specific act of cannibalism would be bathed in suspensions of *G. homari* released during the demolition of the infected animal. Obviously the lobsters must have some barriers against infection or all would succumb in a fairly short time. Wood (1965b) suggested that "it appeared that damage to the integument of the lobster was a pre-requisite for invasion of *Gaffkya* into

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the bloodstream, and that the consumption of infected food by itself did not lead to infection."

Accordingly a series of experiments was carried out to determine what the effective barriers are and to provide an understanding of the mode of transmission of *G. homari*.

METHODS

Bacteriological

The bacteria used in this study were obtained from the American Type Culture Collection (ATCC) and our own culture collection (FRB). The strain of *G. homari* used in the live animal experiments was originally isolated from a diseased lobster by Dr Rabin. The continued virulence of *G. homari* was ensured by continuous passage of the microorganisms through lobsters held at 15 C. Methods used for the isolation and identification of *G. homari* have been described previously (Stewart et al., 1966). Trypticase Soy (TS) broth or agar was used for all microbial work; the incubation temperature was 28 C.

Infective Cell Suspensions

Infective cell suspensions were prepared by inoculating TS broth (Baltimore Biological Laboratory, Baltimore, Md.) with 0.2 ml of hemolymph removed from an infected lobster; the cultures were incubated for 24 hr at 28 C. The *G. homari* cultures were harvested, the cells washed three times with sterile 3% NaCl and suspended for immediate use in sterile 3% NaCl at the desired density, determined at 420 mμ using a Spectronic 20 spectrophotometer (Bausch and Lomb). The suspensions were checked for purity and the viable numbers were determined by the plate count method of Miles and Misra (1938). All lobsters injected were given a standard volume of 1.0 ml/kg of their live weight, injected into the ventral abdominal sinus. The site was swabbed thoroughly with 70% ethanol before and after injection.

Bactericidal Tests

Bacteria under aseptic conditions were harvested, washed, and suspended in sterile 3% NaCl (optical density at 420 mμ of 0.2 equalling approximately 2×10^6 bacteria/ml) and were added to sterile gastric fluid (1 volume of bacterial suspension to 3 volumes of gastric fluid). Samples were taken at various time intervals and the bacterial numbers were determined by the plate count method of Miles and Misra (1938) using TS agar incubated at 28 C. Controls were run in parallel with sterile 3% NaCl substituted for the gastric fluid.

Lobsters

Lobsters (*Homarus americanus*) were obtained immediately after their capture from Eastern Passage, N.S. (average weight, 534 g) and Fourchu, N.S. (average weight, 504 g). Both large chelae were closed with rubber bands unless otherwise specified, and the lobsters were held prior to experimental work, in tanks supplied with a continuous flow of aerated sea water (2 changes/hr), ranging in temperature from 1 to 4 C. All experimental work was carried out in tanks supplied with aerated, recirculated, unfiltered sea water at a constant temperature of 15 C. Individual shelters, which the lobsters could use at will, were provided in all tanks unless otherwise specified. Of the water in the experimental and control tanks, 80% was replaced each Monday, Wednesday, and Friday during the course of the experiments. All lobsters were acclimated to 15 C for a minimum of 2 weeks and were examined and found to be free of *G. homari* before being included in an experiment. None of the lobsters was fed during the acclimation period or the experimental period except as indicated in the in vitro experiments with lobster gastric fluid.

Isolation in Plastic Tubes

Rigid plastic tubes were used when the experiment required lobsters to share the same tank but to have no direct physical contact with one another. Each animal was placed in an open-ended black plastic tube (10 × 25 cm) which had approximately 20 holes (1.5 cm diam), evenly spaced, in the walls. The plastic tube was then inserted into a close-fitting bag made from white nylon netting (approx 0.3 cm mesh size). The open end of the bag was secured by a heavy rubber band leaving only the interior of the plastic tube as free space for the lobster.

DELIBERATE WOUNDING

A standard wounding pattern was adopted. The individual lobster's ventral abdominal membrane was punctured twice in the first abdominal somite and twice in the third with a sterile 17 ga hypodermic needle, and a transverse slit, approximately 1 cm long, was made aseptically in the centre of the ventral surface of the second abdominal somite. All five wounds were deep enough to allow hemolymph to escape freely from all parts of the wounds.

CHELAE PLUGS

Both chelae of each lobster in the group specified were closed with machine-made wooden plugs, the common North American commercial practice described by Wilder and McLeese (1961).

GASTRIC FLUID

Gastric fluid for in vitro work was removed from the live lobsters' stomachs by gentle suction, using a syringe attached to small diameter rubber tubing. The gastric fluid from the required number of lobsters was pooled and centrifuged at 30,000 × *g* for 10 min at 4 C to remove particulate matter. A portion of each pooled sample was used immediately and the remainder lyophilized for use later. The gastric fluid was sterilized in the earlier experiments by passing it through a membrane filter (0.45 $m\mu$ pore size); however, after we discovered that heating did not destroy the fluid's inhibitory power, a modified pasteurization procedure was adopted (75 C for 1 hr).

LYSOZYME

Bacto-lysozyme (crystallized from egg white) and Bacto-lysozyme substrate (killed dried cells of *Micrococcus lysodeikticus*) were used with Bacto-lysozyme buffer in the standard procedure[1] to prepare a standard curve for comparison with the Bacto-lysozyme activity against *G. homari*. The standard lysozyme procedure was modified for the determination of the lytic activity present in lobster gastric fluid or fractions of it; the Bacto-lysozyme substrate (*M. lysodeikticus*) or *G. homari* were employed as substrates with no buffer added and the incubation time was extended as indicated in the results.

GASTRIC FLUID FRACTIONATION

The chromatographic fractionation of the gastric fluid on Sephadex G-75 (Pharmacia Fine Chemicals Inc., New Market, N.J.) was carried out at 4 C using a fraction collector equipped with an ultraviolet monitor recording at 280 $m\mu$. The column (inside diam 25 mm) was filled to a height of approximately 45 cm with Sephadex G-75 prepared previously according to the manufacturer's instructions. After the column had been washed thoroughly it was charged with 7 ml of gastric fluid and run with a pressure head equivalent to the bed height. Fluids used in the preparation and elution of a column were either 0.9% NaCl or distilled water.

RESULTS

WHOLE ANIMAL STUDIES

We showed that *G. homari* does not escape from an intact infected animal during the course of the infection. Five lobsters were placed in plastic open-mesh envelopes which prevented physical contact between them and each was injected with 0.5 ml of a *G. homari* suspension (6.4×10^6 bacteria/ml); we were unable to detect *G. homari* in the tank water (recirculated but unfiltered) up to the time of the death of the lobsters.

The data on the introduction into lobsters of the pathogen by the oral route as compared with direct injection are summarized in Table 3. An extension of this work included an examination of the contents of the stomachs of five of the lobsters in group 8. The intact stomachs were exposed surgically and opened under aseptic conditions at the end of the 52-day observation period; the stomach contents were streaked on TS agar plates and incubated for 4 days. *Gaffkya homari* was not detected. Four other microbial types, however, were found: (1) transparent, pin-point colonies of gram-positive, large, pleomorphic rods; (2) large, yellow, mucoid colonies of slender, gram-positive rods; (3) pinkish-white, powdery colonies possessing an earthy odor (an actinomycete-like organism); and (4) large, spreading, grey, mucoid colonies of gram-positive cocco-baccilli. These organisms when streaked on a TS agar plate at right angles to a freshly seeded streak of *G. homari* produced no inhibition of the growth of *G. homari* during 4 days incubation at 28 C.

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[1] Difco Supplementary Literature, 1962. Difco Laboratories, Detroit, Michigan.

The disappearance of *G. homari* from the stomachs of the lobsters and the lack of infection led to an in vitro examination of the gastric fluid.

In Vitro Studies

The lobster gastric fluid was bactericidal for the various strains of *G. homari* tested (Fig. 1*a*). The effect on representatives of several other bacterial genera is shown in Fig. 1*b*. The *p*H of the gastric fluid did not change during the incubation period.

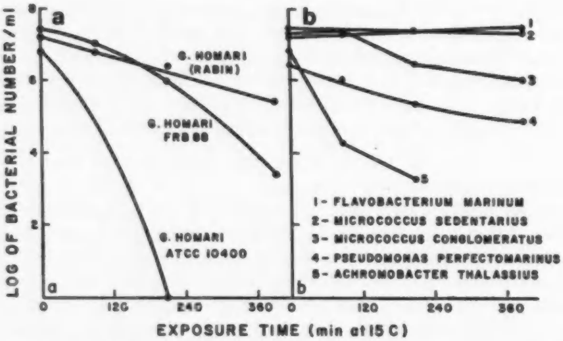

FIG. 1. Bactericidal effect of lobster gastric fluid during incubation with: (*a*) *Gaffkya homari* strains; (*b*) representatives of other bacterial genera.

Periodic microscopic examinations of a mixture of gastric fluid and *G. homari* during a 144-hr incubation period revealed that the bacteria were intact long after the mixture became sterile. The bacteria gradually lost their gram-positive properties until by the 136th hr almost all were gram negative. The bacteria gradually disappeared during the course of the incubation; small amounts of cell debris accumulated and in turn disappeared.

These observations plus the fact that the normal lobster gastric fluid has *p*H values ranging from 4.7 to 5.0 indicated that acidity rather than a lysozyme was the probable cause of the inhibitory effect.

The comparative resistance of *G. homari* to lysis was demonstrated by the use of egg white lysozyme. Concentrations of the enzyme ranging from 0.001 to 0.01 mg/ml caused appreciable lysis of *M. lysodeikticus* at all levels in 20 min and essentially complete lysis at the strongest concentration. A minimal degree of lysis occurred with *G. homari* only at the strongest concentration after 60 min incubation. When a very high level of lysozyme (10 mg/ml) was employed, complete lysis of *G. homari* occurred within 20 min.

Some, but not all, of the gastric fluid samples exhibited a weak lytic effect against *M. lysodeikticus* (observable after prolonged incubation) but none against *G. homari* despite a 6-hr incubation period. All of the gastric fluid samples, however, were bactericidal for *G. homari*.

Fractionations on Sephadex G-75 of gastric fluids possessing lytic activity for *M. lysodeikticus* were performed. When fractions 5, 9, 11, 19, and 27 (Fig. 2) were incubated with *G. homari* under the standard test conditions for 420 min, all were sterile at the end of the incubation period. The buffering capacity, protein, and *p*H values for this fractionation are illustrated in Fig. 2. The bulk of the lytic activity was apparent in fractions 5 through 17 (Fig. 3). One column fractionation of a sample of gastric fluid was continued up to fraction number 150 (1500 ml of eluant) to determine whether any abrupt changes in *p*H occurred due to the presence of small, free, highly acidic molecules. In all of

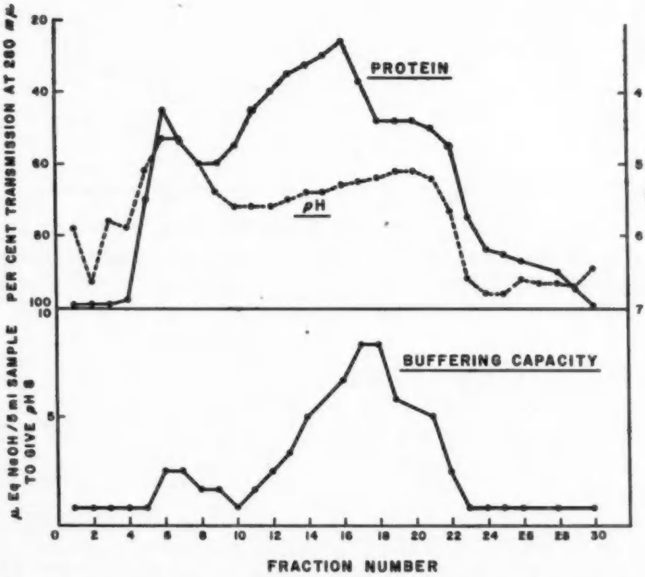

FIG. 2. Sephadex G-75 gel filtration of gastric fluid possessing lytic activity *Micrococcus lysodeikticus*. Distilled water as eluant; 10-ml fractions.

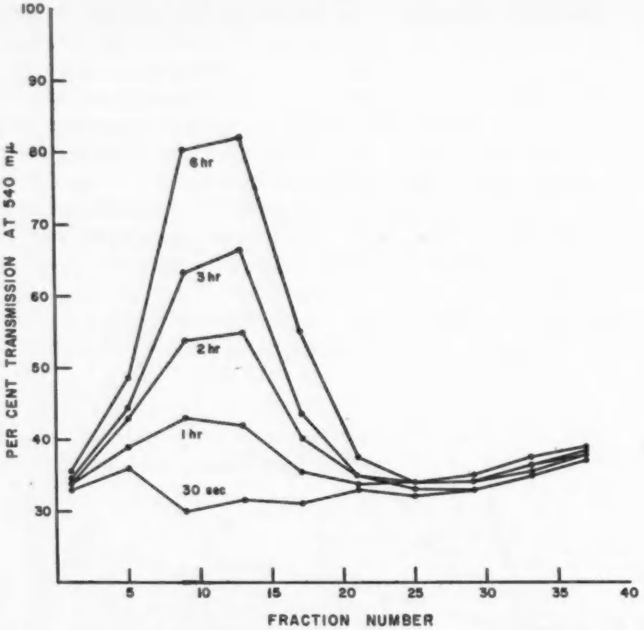

FIG. 3. Elution of principle lytic for *Micrococcus lysodeikticus* from Sephadex G-75. Distilled water as eluant; 10-ml fractions. Times refer to sequential readings on the same samples for fractions incubated with Bacto-lysozyme substrate (*M. lysodeikticus*).

these later fractions the *p*H remained close to 6, the approximate *p*H of the distilled water eluant; the titratable acidity remained negligible. Apparently the acidity of the gastric fluid is a function of its protein molecules maintained within a narrow *p*H range instead of being the result of an excess of small free acidic molecules.

The implication of the acidity as the factor solely responsible for the bactericidal effect was confirmed by adjusting the *p*H of samples of gastric fluid with 0.1 N HCl or NaOH to give a range of *p*H values. The results of repeated determinations are illustrated in Fig. 4. Essentially identical results were obtained when veronal or sodium acetate buffers at these same *p*H values were substituted for gastric fluid in the bactericidal tests. When fractions 14 and 15 (see Fig. 3) were combined and tested over a relatively wide range of *p*H values the results were consistent with the results presented in Fig. 4. The bactericidal effect increased with increasing acidity.

Enzymes (protease, lipase, and β-glucosidase) known to be present in the gastric fluid (unpublished results) were not involved in the bactericidal effect. Heating the gastric fluid at 75 C for 60 min destroyed these enzymes' activities but left the bactericidal activity intact. Boiling the gastric fluid for 15 min

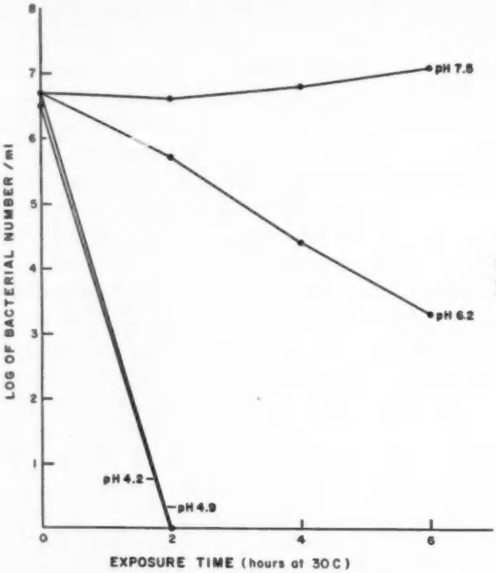

FIG. 4. Effect of bactericidal activity of adjusting gastric fluid *p*H values. Test organism was *G. homari* (Rabin).

did not alter the *p*H value or the fluid's inhibitory effect against *G. homari*. Interestingly no precipitate formed upon boiling the gastric fluid at its normal *p*H value, although the gastric fluid has a high protein concentration (6–10%). Lyophilization of the gastric fluid did not alter the bactericidal activity or the *p*H value of the reconstituted material.

Gastric fluid from lobsters starved for 2 weeks at 15 C had the same *p*H value and bactericidal effect against *G. homari* as that from lobsters kept on a quantitatively adequate diet of beef liver alternating with sardine herring.

The total bactericidal effect of the gastric fluid did not vary with temperature but the rate was temperature-dependent. When the incubation of *G. homari* with gastric fluid was carried out at three different temperatures (7.5, 15, and 25 C) the numbers of bacteria surviving 240 min at 15 C was 10% of those surviving at 7.5 C. Those surviving at 25 C were less than 1% of the numbers surviving 240 min at 15 C.

DISCUSSION

Most of the experimentally infected lobsters in direct contact with uninfected animals (Table 1, groups 1 and 2) were partially eaten at or close to the time of death by their healthy fellow lobsters. Large numbers of *G. homari* are released into the surrounding water by this event providing an inoculum for transfer to the uninfected animals (the hemolymph of moribund, gaffkemic lobsters will contain approximately 10^9 *G. homari*/ml (unpublished results)). Although all of the lobsters' chelae were closed with rubber bands the fighting

TABLE 1. Exposure of healthy lobsters to infected animals.

Group	Treatment[a]	Inoculum, *G. homari*/ml	Initial no. of lobsters	No. dead	No. of dead infected[b]	Observation period (*days*)	Mean time to death for infected lobsters (*days*)
1	(*a*) Injected SV[c] (= 1.0 ml/kg of live wt of lobsters) of live *G. homari* into VAS[c]	7×10^6	10	10	10	49	13 ± 1.4[d]
	(*b*) No injection		10	5	5	49	23 ± 4.2
K-1[e]	(*a*) Injected SV of sterile 3% NaCl into VAS	0	10	0		49	
	(*b*) No injection		10	0		49	
2	(*a*) Injected SV of live *G. homari* into VAS	4×10^6	10	10	10	35	12 ± 1.7
	(*b*) No injection		10	9	9	35	26 ± 1.6
K-2	(*a*) Injected SV of sterile 3% NaCl into VAS	0	10	0		35	
	(*b*) No injection		10	0		35	
3	(*a*) Injected SV of live *G. homari* into VAS (lobsters in individual rigid plastic tubes, covered with nylon net)	13×10^6	10	10	10	31	10 ± 1.0
	(*b*) No injection (lobsters in plastic tubes covered with nylon net) *a* and *b* kept in same tank		10	0		31	
K-3	Injected SV of sterile 3% NaCl VAS (lobsters in plastic tubes covered with nylon net)	0	10	0		31	

[a] All lobsters were kept in aerated recirculated sea water at a temperature of 15 C throughout the experiments. An individual tank was used for each experimental group; this was well separated from its corresponding control tank. The *a* and *b* lobsters in groups 1, K-1, 2, and K-2 were allowed to mingle freely in their respective tanks which were not supplied with shelters.

[b] None of the survivors was infected.

[c] SV = standard volume; VAS = ventral abdominal sinus.

[d] Standard error.

[e] K = control.

and struggling presumably produced wounds which served as portals of entry for *G. homari*. The mean time to death for the initially uninfected lobsters was twice that of the deliberately infected animals, and it is probable that the former became infected at approximately the time of death for the deliberately infected. Chance alone, modified by the individual lobsters' behavioral pattern, would appear to govern the incidence of wounding since in group 2*b*, 90% of the lobsters not infected initially succumbed, compared with only 50% of those in identical circumstances (group 1*b*).

Transmission of gaffkemia did not take place when infected lobsters and healthy unwounded lobsters were all enclosed in individual plastic tubes in the same tank (group 3; Table 1); the retention of *G. homari* by intact infected lobsters would eliminate the possibility of an infection even if the healthy animals had been wounded. The infected animals (group 3*a*) died in a shorter period of time than usual. None of the lobsters in group 3*b* or K-3 died, indicating that the confinement was not unduly severe.

Proof that a rupture of the integument is essential for infection is illustrated by the data in Table 2. Only one of the unwounded lobsters (group 4) acquired an infection; presumably it had a small wound which went undetected. The majority of those wounded immediately prior to immersion in the *G. homari* suspension became heavily infected. Surprisingly two of these (group 5*a*) were not infected indicating an extremely rapid wound-sealing mechanism.

Further evidence of the effectiveness of the wound-sealing mechanism, despite relatively severe wounding, is apparent in the results for group 5*b*. Only one lobster became infected when this group was immersed in the *G. homari* suspension 24 hr after they had been wounded; this lobster's transverse wound was still poorly closed at the time of death.

It was apparent that the wooden plugs used to close the chelae of lobsters (group 6) produced a wound which did not heal as well as wounds caused by other means (group 5). All of those plugged immediately prior to exposure to *G. homari* succumbed to the infection and more than half of those whose chelae were plugged 24 hr prior to the exposure also acquired the infection. Obviously the commercial use of plugs for closing chelae can be a major contributing factor to the massive outbreaks of gaffkemia experienced in the lobster industry.

The results in Tables 1 and 2 suggest an explanation for the observations made by Wilder and McLeese (1961) in their comparison of different methods for inactivating lobster chelae. At the beginning of the experiment they exposed two groups of lobsters to dead lobsters infected with *G. homari*. Of the group whose chelae were closed with wooden plugs, 91% were dead by the 16th day of the experiment. The mortalities in the other group (whose chelae were closed with rubber bands) totalled 78% but were distributed fairly evenly from the 3rd day of the experiment to the 46th day.

Our previous studies (Cornick and Stewart, 1968a) indicate that at the temperatures employed by Wilder and McLeese (1961) their animals should have died within a 16-day period after infection. Thus their lobsters with the plugged chelae would appear to have acquired the infection early and more or

TABLE 2. Immersion of wounded and unwounded lobsters in suspensions of live *Gaffkya homari*.

Group	Time of treatment[a] All lobsters enclosed in individual plastic tubes covered with nylon netting. Heavy suspensions of *G. homari* in 3% NaCl mixed with the experimental tank waters at exposure time.	Initial inoculum, *G. homari*/ml[b] of tank water	Initial no. of lobsters	No. dead	No. of dead infected[c]	Observation period (*days*)	Mean time to death for infected lobsters (*days*)
		Unwounded					
4		1.8×10^6	10	1	1	36	17
K-4[d]		0	10	0		36	
		Deliberately wounded					
5*a*	Immediately prior to exposure	1.8×10^6	10	9	8	36	15±1.9
5*b*	24 hr prior to exposure	1.8×10^6	10	2	1	36	18
K-5	At exposure time	0	10	2	0	36	
		Both chelae closed with wooden plugs					
6*a*	Immediately prior to exposure	1.8×10^6	10	10	10	36	13±2.4
6*b*	24 hr prior to exposure	1.8×10^6	10	8	6	36	16±4.0
K-6	At time of exposure	0	10	3[e]	0	36	

[a] All lobsters were kept in aerated recirculated sea water at a temperature of 15 C throughout the experiments.

[b] *Gaffkya homari* numbers/ml of tank water had been reduced to 8×10^5/ml at the end of the first 24-hr period; the tank water was replaced with new sea water 24 hr after introduction of inoculum. The controls (K) were kept in separate tanks and were not exposed to *G. homari*.

[c] None of the survivors was infected at the end of the observation period.

[d] K = control.

[e] Partially molted in the plastic tubes and died because of confined space.

less simultaneously, i.e. possibly at the time of the introduction of the dead lobsters. The most likely portal of entry would be the wounds in the chelae caused by the wooden plugs. The data for their other lobster group (chelae closed with rubber bands) suggest that very few of these animals acquired the infection initially but rather that the transmission of the pathogen to a few lobsters at a time occurred continuously throughout the course of the experiment.

Our results and those of Wilder and McLeese (1961) prove that the closing of the chelae with wooden plugs ensures that a simultaneous transmission of *G. homari* to a high percentage of the lobsters will occur when the pathogen is present, especially under crowded commercial conditions and particularly if the exposure occurs within a short period after the plugging. The wounds caused by plugs are undoubtedly aggravated by the plug moving in the wound as a result of external pressures.

Closing the chelae with rubber bands does not result in simultaneous transmission to large numbers of animals but it does not prevent all wounding, nor can it prevent cannibalism. Consequently, when lobsters are banded, a long-term continuous-type of transmission can still occur.

Another type of wound which can occur when the chelae are closed securely are those inflicted on the soft under parts of the lobster by the spiny projections of other lobsters. The frequency and severity of this wounding are exacerbated by the lobsters' aggressive behaviour and propensity for heaping themselves in a squirming mass in the darkest corner of the tank. Lobsters held under crowded conditions also eat each other's antennae thereby producing another portal of entry.

The data in Table 3 demonstrate that *G. homari* introduced into the stomach of healthy lobsters does not result in an infection. In contrast those infected by injections from the same suspension of *G. homari* all died within the time expected for this temperature (Cornick and Stewart, 1968a) proving that the virulence of the strain was unchanged from the previous work. These determinations show that feeding lobsters with infected material (Snieszko and Taylor, 1947; Wood, 1965b; Rabin and Hughes, 1968) would not be expected to produce an infection in intact animals.

An explanation and a confirmation of the in vivo results were provided by the in vitro experiments with gastric fluid. The continued presence of the bactericidal activity regardless of the lytic activity in the gastric fluid, the even distribution of the bactericidal activity throughout the void volume of the Sephadex G-75 column (fractions 5, 9, 11, 19, and 27; Fig. 2) and the concentration of the lytic activity in the early fractions eluted from the Sephadex column (Fig. 3) demonstrate the noninvolvement of a lysozyme. The fact that changing the *p*H value of the gastric fluid (Fig. 4) or fractions of it altered the bactericidal effect indicates the role of acid conditions; achievement of the same effect with buffers demonstrates the protective effect conferred by the acidity of the gastric fluid. The disappearance of *G. homari* from the stomachs of lobsters

TABLE 3. Comparison of infection routes: direct injection vs. oral introduction of *Gaffkya homari*.

Group	Treatment[a]	No. of *G. homari*/ml of inoculum	Initial no. of lobsters	No. dead	No. dead infected[b]	Observation period (*days*)	Mean time to death for infected lobsters (*days*)
7	Injected SV[c] of live *G. homari* into VAS[c]	5×10^6	10	10	10		22 ± 1.9
K-7[d]	Injected SV of sterile 3% NaCl into VAS	0	10	0		52	
8	Introduced SV of *G. homari* into stomachs of lobsters through catheter tubing inserted into the stomach via the oral cavity and esophagus	5×10^6	10	0		52	
K-8	Introduced SV of sterile 3% NaCl into stomachs of lobsters as above	0	10	0		52	

[a] All lobsters were kept in aerated recirculated sea water (with shelters) at a temperature of 15 C throughout the experiments.
[b] None of the survivors was infected at the end of the observation period.
[c] SV = standard volume; VAS = ventral abdominal sinus.
[d] K = Control.

after its introduction by catheter (group 8) and the lack of infection subsequently, are entirely consistent with this conclusion.

The destruction of the gastric fluid's digestive enzyme activity by heat without altering the bactericidal effect demonstrated that these enzymes played no part in killing *G. homari*. These and other enzymes are probably responsible for the eventual disintegration of the dead bacteria.

The *p*H of the lobster gastric fluid does not change with the consumption of food, and the rate of clearance of food from the stomach increases with increasing temperature; lobsters kept at 15 C require at least 3 days to clear their stomachs of food after a meal (unpublished results). This retention time ensures that *G. homari* would be maintained at *p*H 4.7 to 5.0, long enough for destruction, and that infection by *G. homari* via the oral route is adequately guarded against, regardless of the degrees of resistance exhibited by the different strains of *G. homari* (Fig. 1*a*). In comparison other organisms are not so susceptible (Fig. 1*b*).

The reasons for massive outbreaks of gaffkemia among lobsters in the commercial holding units where close contact is enforced become apparent once the mode of transmission of *G. homari* is understood. The epidemiology of the disease in nature, however, is not understood; information on the extent and effect of the disease in the natural population is lacking. Two reports (Rabin, 1965; Stewart et al., 1966) provide data on the ubiquity of *G. homari* in the lobster population and show that occasionally high percentages of the lobsters are infected at the time of capture.

In nature the lobster is a solitary animal; consequently the transmission of the disease from one lobster to another would not be expected to occur on the massive scale noted in commercial holding units. The fact that massive infections in nature have been noted raises the possibility of a vector such as *Cancer irroratus* (Cornick and Stewart, 1968b). This crab can carry *G. homari* for long periods with no apparent ill effect. The muds and debris on the lobster grounds are other possible sources of *G. homari*. Wood (1965a) has shown that *G. homari* will live in sea water supplemented with lobster hemolymph and Goggins and Hurst (MS, 1960) have shown that it will live and multiply in the slimes coating underwater lobster equipment and in the muds of lobster pounds. A unique deposit of the right kind of organic debris on the ocean floor together with conditions favorable to *G. homari* could give rise to a flourishing population of the pathogen. Under these circumstances wounds caused to the lobsters by predator or prey, infected or not, would result in a high infection incidence.

ACKNOWLEDGMENTS

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Variants of Lactate Dehydrogenase Isozymes in Sera of Sockeye Salmon (*Oncorhynchus nerka*)

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Hodgins, H. O., W. E. Ames, and F. M. Utter. 1969. *Variants of lactate dehydrogenase isozymes in sera of sockeye salmon* (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 26: 15–19.

Three phenotypes of lactate dehydrogenase (LDH) isozymes were found in sera of sockeye salmon (*Oncorhynchus nerka*), presumably representing B′B′, B′B, and BB genotypes. No association was obvious between LDH phenotype of sera and sex or total body length.

Of 1006 sera from Asian, Bristol Bay, and Gulf of Alaska stocks, 826 were B′B′ and 180 were B′B or BB. Of 591 sera from Washington and British Columbia stocks, 589 were B′B′ and 2 were B′B; both of the B-allele phenotypes were found in fish captured at the Skeena River in northern British Columbia. These findings suggested that LDH isozymes should be useful in studies on ocean distribution of sockeye salmon and in characterizing certain Asian and Alaskan sockeye salmon populations.

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INTRODUCTION

Multiple forms of lactate dehydrogenase (LDH) enzymes (isozymes) have been extensively examined in many animal species. Where studied, these isozymes are tetramers. In mammals, LDH patterns are generally alike; that is, five principal isozymic forms are found that are tetramers of all possible combinations of two monomers frequently designated A and B (Cahn et al., 1962; Boyer et al., 1963; Markert, 1963, 1965; Shaw, 1964; Kaplan, 1964; Salthe et al., 1965). Several mammals and birds synthesize another polypeptide monomer which forms active enzymes in the testes (Blanco and Zinkham, 1963; Blanco et al., 1964; Zinkham et al., 1963, 1964).

Several studies of LDH isozymes in fish have been reported (Market and Faulhaber, 1965; Nakano and Whiteley, 1965; Goldberg, 1965, 1966; Hochachka, 1966; Morrison and Wright, 1966; Odense et al., 1966; Arnheim et al., 1967; Massaro and Markert, 1968). Markert and Faulhaber (1965) examined 30 species and found that they possessed one, two, three, or five major LDH isozymes. Additional LDH isozymes have been found in the eye, skeletal muscle, and other tissues of some species (Markert and Faulhaber, 1965; Hochachka, 1966; Morrison and Wright, 1966; Massaro and Markert, 1968). Morrison and Wright (1966) demonstrated by controlled matings the autosomal transmission of codominant alleles for the subunit in brook trout (*Salvelinus fontinalis*) analogous to B in mammals. The same authors reported that matings of lake trout (*S. namaycush*) × lake trout and of lake trout × brook trout indicated that the A subunit is similarly inherited.

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In this report we describe LDH isozyme variants in the sera of sockeye salmon. We also present gene frequencies calculated for populations of sockeye salmon from different areas.

MATERIALS AND METHODS

Most of the sera tested for LDH isozymes were from immature and mature sockeye salmon collected in marine, estuarine, river, and lake areas of Alaska, British Columbia, and Washington. A pool of sockeye or chinook salmon sera with isozymes of known electrophoretic mobilities was routinely included in tests as a standard control.

Some of the isozymes came from sockeye salmon sera which had been frozen for as long as 6 years. We found that freezing and thawing of whole salmon sera did not markedly alter LDH patterns. We noted, however, that discrete bands of LDH activity could not be resolved from a few of the long-frozen sera and that two weak extra bands of LDH enzyme activity frequently were present anodal to LDH 1, particularly in older samples. After comparing the samples containing extra bands with fresher samples, we concluded that different LDH phenotypes could usually still be discerned.

Sera from three populations of sockeye salmon reared at our laboratory were also tested. These fish were obtained in 1965 as eyed eggs from the Kamchatka River in eastern Siberia, USSR; the Babine River, a tributary of the Skeena River, in British Columbia, Canada; and the Wenatchee River, a tributary of the Columbia River, in the State of Washington. The embryos were transported to the National Fish Hatchery at Leavenworth, Wash., for hatching; taken as fry to the Bureau of Commercial Fisheries (BCF) Laboratory in Seattle, Wash., for freshwater rearing; and transferred at 2 years of age to a large saltwater pond at the BCF Marine Station at Bowman Bay, Wash. Some fish were also maintained in a freshwater tank for comparative studies. These fish were all fed the same commercial food and were maintained under identical or similar environmental conditions.

Blood sera or, in a few instances, plasma were stored either in liquid nitrogen (–196 C) or in a freezer at –15 C and thawed at room temperature before electrophoresis.

Muscle samples were collected simultaneously with sera from marine fish and frozen in a shipboard freezer. On the day of testing, these samples were thawed at room temperature and homogenized at 0 C in equal volumes of phosphate-buffered physiological saline (*p*H 7.4) or distilled water. Homogenates were then incubated in the refrigerator for 1–2 hr and centrifuged at 700 × *g* for 15 min in a refrigerated centrifuge; supernatant fluids were studied by electrophoresis.

Horizontal electrophoresis in starch gel was used to resolve bands of isozymes. Gels consisted of 30 g starch plus 30 ml of a lithium–borate buffer[1] and 80 ml of a Tris–citrate buffer[2] in a total volume of 250 ml. The lithium–borate buffer was the electrode compartment buffer. A voltage of 300 was applied for 10 min, sample wicks were removed, and 400 v were applied until the visible ion front reached a point 9.0 cm anodal to the origin; this required a running time of about 2 hr. During electrophoresis, gels were cooled either in a refrigerator or by ice packs on glass plates placed on top of the gels for protection.

After electrophoresis, zones of LDH activity were made visible by incubating the gels in the dark for 20–60 min at 30 C in the following reaction mixture: lithium lactate (0.5 M), 7 ml; nicotinamide adenine dinucleotide (NAD), 10 mg; phenazine methosulfate, 3.5 mg; *p*-nitro blue tetrazolium, 7 mg; Tris-HCl buffer (0.1 M, *p*H 8.3), 27 ml. Controls on the specificity of staining were run by elimination of substrate or NAD.

NATURE OF LDH VARIANTS

The three LDH phenotypes detected in sockeye salmon sera are shown in Fig. 1. Although random combination of three types of subunits (B, B′, and A)

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[1] 1.2 g lithium hydroxide and 11.8 g boric acid in 1 liter (*p*H 8.2).

[2] 8.66 g Tris and 4.84 g citric acid in 1 liter (*p*H 6.6).

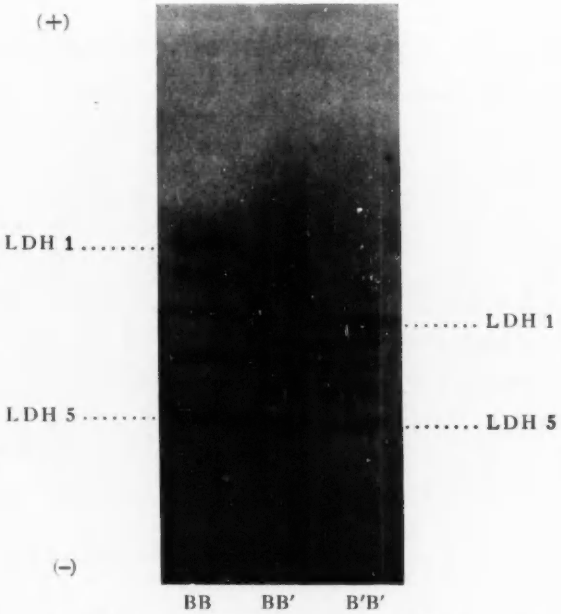
(+)
LDH 1
LDH 5
(−)
LDH 1
LDH 5
BB
BB′
B′B′

Fig. 1. Bands in starch gel illustrating lactate dehydrogenase phenotypes in serum of sockeye salmon.

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would justify prediction of 15 LDH isozyme bands in B′BAA heterozygote sera, the maximum observed was only 9. The same maximum number of bands for heterozygotes was observed by Morrison and Wright (1966) in brook trout and by Goldberg (1966) in lake trout. Possible explanations include overlapping of isozymes, nonrandom combination of subunits, and nonresolution of weak isozymes.

One (Kamchatka River) of three populations of sockeye salmon reared from embryos to more than 2 years of age in very similar or identical controlled environments demonstrated more than one phenotype of serum LDH (Table 1); this is consistent with the concept that these phenotypes directly reflect genotypes and are not the result of differential environmental effects.

TABLE 1. Frequencies of lactate dehydrogenase (LDH) phenotypes in sera of sockeye salmon from seven populations.

	LDH phenotypes observed			Allele frequency		LDH phenotypes expected			
Fish origin	B′B′	B′B	BB	B′	B	B′B	B′B	BB	*P* of chi-square
Babine R., B.C.[a]	143	0	0	1.000	0	143	0	0	–
Wenatchee R., Wash.[a]	85	0	0	1.000	0	85	0	0	–
Kamchatka R., Siberia[a]	85	15	1	0.916	0.084	84.7	15.6	0.7	>.050
Tazlina L., Alaska	42	5	1	0.927	0.073	41.2	6.5	0.2	>0.05
Gulkana R., Alaska	22	7	0	0.879	0.121	22.4	6.1	0.4	>0.30
Bering L., Alaska	26	11	1	0.829	0.171	26.1	10.8	1.1	>0.80
American R., Alaska	13	8	1	0.773	0.227	13.2	7.7	1.1	>0.90

[a]Obtained as eyed eggs and reared in the laboratory.

No association was obvious between serum LDH phenotype and sex or total body length for 244 salmon taken from the North Pacific Ocean (on which we have sufficient sampling data). Homology was apparent for all three phenotypes between LDH isozymes in sera and skeletal muscle extracts from the same individuals, although, as reported for other salmonid fishes (Hochachka, 1966; Morrison and Wright, 1966; Massaro and Markert, 1968), additional LDH isozymes are also present in muscle.

FREQUENCY DISTRIBUTION OF LDH VARIANTS AND THEIR POTENTIAL APPLICATION FOR DEFINING AND CHARACTERIZING POPULATIONS

In Table 1 are shown the frequencies of serum LDH phenotypes of the three laboratory-reared populations of sockeye salmon and of four wild populations representative of a range of phenotypic frequencies. None of these populations differed significantly in its phenotypic distribution from the binomial distribution expected for two alleles in accordance with the Hardy–Weinberg law (Hardy, 1908; Stern, 1943).

The B-allele phenotypes appear in much greater frequency in samples from Alaskan and Asian waters than they do in those from southern British Columbia and Washington state (Table 2). We have not detected a B′B or BB phenotype in 418 sera from sockeye salmon captured south of the Skeena River, and only 2 of 173 fish from the Skeena River area contained the B-allele (both B′B heterozygotes). Conversely, in tests of 581 sera we have not failed to detect one or both of the B-allele phenotypes in sockeye salmon stocks from rivers and lakes in Siberia and Alaska; phenotypes of the B allele were also frequently present in sera from 425 marine fish from Bristol Bay and the northern Gulf of Alaska. If further testing confirms this distribution, LDH isozymes may be useful indicators of ocean intermingling and separation of Asian–Alaskan from British Columbia–Washington sockeye salmon. Even if this separation proves not possible, the ratios of phenotypes of B and B′ alleles are shown by the present data to vary considerably; their frequencies should be useful in defining and characterizing certain populations.

TABLE 2. Distributions of lactate dehydrogenase (LDH) phenotypes of sockeye salmon sera grouped by geographical area of fish capture.

Fish origin	No. of fish tested	% LDH phenotype		
		B′B′	B′B	BB
Kamchatka R., Siberia	101	84.2	14.8	1.0
Bristol Bay and northern Gulf of Alaska rivers, lakes, and estuaries	480	80.4	18.3	1.3
Bristol Bay and Gulf of Alaska — marine	425	83.5	15.5	1.0
B.C. and Washington rivers, lakes, and estuaries	591	99.7	0.3[a]	0

[a]These fish (two) were from the Skeena River area of the northern British Columbia coast.

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Responses of Yearling Smallmouth Bass (*Micropterus dolomieui*) to Artificial Shelter in a Stream Aquarium[1,2]

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HAINES, TERRY A., AND ROBERT L. BUTLER. 1969. *Responses of yearling smallmouth bass (Micropterus dolomieui) to artificial shelter in a stream aquarium.* J. Fish. Res. Bd. Canada 26: 21–31.

Juvenile smallmouth bass showed a marked response of utilizing opaque plastic coverts in a stream aquarium with two water velocities (12 and 28 cm/sec). The most complex of six opaque coverts at the higher water velocity was occupied a mean time of 7190 ± 6 out of 7200 sec. The presence of a visual reference point was the first feature to attract a fish to a covert for a marked period of time. The addition of an area of darkness increased the use of a covert. Maximum use was obtained with the above features plus an area of quiet water. Clear plastic coverts, which supplied only thigmotactic stimuli or thigmotactic stimuli and an area of quiet water, were rarely used.

Received March 12, 1968

INTRODUCTION

THIS STUDY concerns the importance of a visual reference, a thigmotactic reference, an area of darkness, and an area of quiet water in use of coverts by juvenile smallmouth bass. The features of coverts which make them attractive to fish were previously examined only briefly (Breder and Nigrelli, 1938; Carlisle et al., 1964; Hartman, 1963).

MATERIALS AND METHODS

A stream aquarium with a side observation window was used in the study. Lighting and water velocity in the aquarium were controlled, but not water temperature. Fish were individually exposed to six types of opaque coverts and five clear plastic coverts, each at two water velocities. Utilization of each covert by each fish was continuously recorded for 2 hr.

STREAM AQUARIUM

Water for the aquarium was obtained from a small waterfall located on Cedar Run, Linden Hall, Pennsylvania. It reached the aquarium by gravity feed through a 20.3-cm aluminum

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[1] Based on a thesis submitted by T.A.H. in partial fulfillment of the requirements for the degree of Master of Science from Graduate School, Pennsylvania State University, University Park, Penn.

[2] Contribution from Pennsylvania State University, Agriculture Experiment Station, Project 1757, Paper 3343.

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[5] Cooperating Agencies: U.S. Bureau of Sport Fisheries and Wildlife, Pennsylvania Fish Commission, and Pennsylvania State University.

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irrigation pipe. Water temperatures during the study period ranged from 7 to 18 C. The water was alkaline, with a *p*H of 8.2 and total alkalinity as $CaCO_3$ of 105 ppm. All water was passed through a bag net, mesh size 0.5 mm, which excluded most drift organisms from the aquarium.

Water velocity in the aquarium was controlled by means of a butterfly valve on the intake pipe and a sliding Plexiglas door on the outlet end of the aquarium. A plexiglass deflector plate and five hardware cloth screens, 6-mm mesh, reduced the turbulence of the incoming water. The water velocity, as measured throughout the aquarium at mid-depth with a Gurley Pigmy current meter, averaged 12 cm/sec at low velocity and 28 cm/sec at high velocity. The velocity at mid-depth in the sections used for covert placement ranged from 8.5 to 11 cm/sec at low velocity and from 20 to 24 cm/sec at high velocity. At no time did these velocities cause the fish to be forced against the lower screens of the aquarium.

The aquarium (Fig. 1), which was constructed of 16-mm plywood and painted a flat brown, was 200 × 60 × 40 cm high. The observation area was 120 × 60 cm and had a Plexiglas window, 114 × 20 cm, set flush in one side of the aquarium. Fish were confined to the observation area by hardware cloth screens at the upper and lower ends of the area. The observation area was divided into 18 sections, each 20 cm square, by placing black dots (6-mm diam) on the bottom at the intersections of the squares. The sections were numbered and used for locating fish in the observance area.

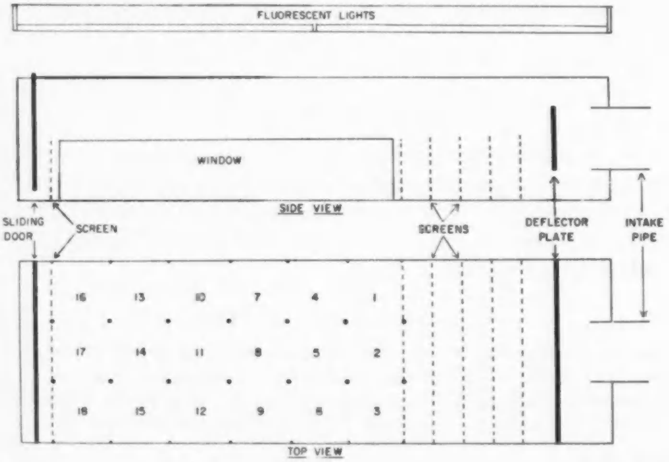

FIG. 1. Arrangement of stream aquarium, lighting, and water level in studies of smallmouth bass behavior. The sites for placement of coverts and location of fish are numbered in the top view.

The observation area was lighted by four 40-w fluorescent lamps, arranged in two groups, placed end to end, and suspended 55 cm over the water surface. Light intensity at the water surfaces ranged from 160 to 220 ft-c, with the corners being of lowest intensity. There were no shadows. An electric timer turned the lights on at 8:00 AM and off at 8:00 PM.

The aquarium was enclosed in a covered platform and screened off with black polyethylene sheeting to exclude extraneous light. With this arrangement the area outside the observation window was dark in relation to the interior of the aquarium, and movements outside the window did not distrub the fish.

Artificial Coverts

The coverts were designed to supply various combinations (Table 1) of the features thought to be important as shelter for fish, i.e., a visual reference, a tactual reference, an area of darkness, and an area of quiet water. They were constructed of 6-mm plexiglass and were 20 cm square × 5 cm high. Two coverts of each of five types were constructed (Fig. 2), one being left clear and the other painted flat black. The sixth type, a bottom plate, was constructed in black only, as a clear bottom plate did nothing to modify the bottom of the aquarium. Legs were made of 9-mm diam wood dowels. The current was from right to left (Fig. 2).

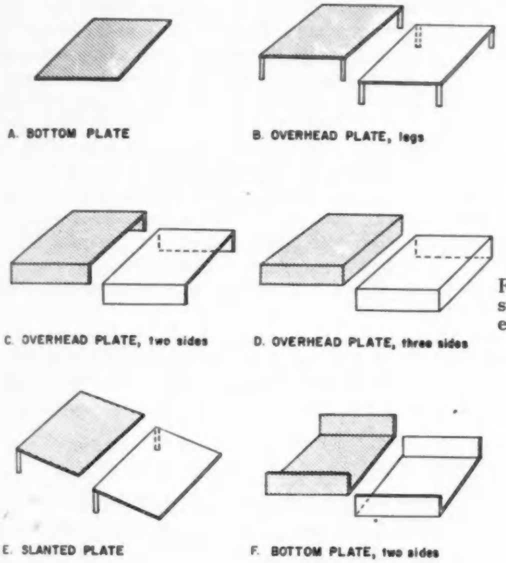
A. BOTTOM PLATE
B. OVERHEAD PLATE, legs
C. OVERHEAD PLATE, two sides
D. OVERHEAD PLATE, three sides
E. SLANTED PLATE
F. BOTTOM PLATE, two sides

FIG. 2. Types of coverts tested in the stream aquarium. Coverts were oriented with the current flowing from right to left.

Fish

Fish used in the study were wild smallmouth bass, *Micropterus dolomieui* Lacépède, obtained by electrofishing from Bald Eagle Creek, near Julian, Pennsylvania. The fish were anesthetized in M.S. 222 (Sandoz Chemical Co.), weighed, measured, and distinctively finclipped. Aged as yearlings they had an average length of 11.6 cm with a range from 10.2 to 13.2 cm.

Between trials fish were held in a live-holding box placed in the stream adjacent to the aquarium building. The box was subject to natural light, not the timed fluorescent lights of the aquarium.

During the experiment two fish were lost and replaced with other fish held in reserve. The losses occurred in the holding box between the low and high velocity experiments; therefore the continuity of each experiment was not interrupted. Near the end of the observations additional fish were needed for clarification of doubtful observations. These fish were obtained by the same means and from the same place as the original fish.

Methods

A 20-pen event recorder (Esterline Angus Instrument Co., Inc., Box 24000, Indianapolis, Indiana) was used to make the record of covert utilization. A keyboard was constructed containing 18 push buttons, corresponding to the 18 sections in the aquarium. The section occupied by a fish at any one time was considered that section directly under its head. The chart at the end of each trial contained a record of time spent in each area of the aquarium.

Positions 8, 11, and 14, in the middle of the aquarium, were selected for placement of the coverts. Preliminary observations indicated that these positions were the least occupied areas of the aquarium when no coverts were available.

The experiments consisted of a series of 2-hr (7200-sec) recordings of covert-seeking responses of the fish. A single fish was placed in the observation area of the aquarium and allowed to adjust to the surroundings for 24 hr. A 2-hr record was obtained of the time spent in the various sections of the aquarium with no coverts. Coverts were then presented in the order listed in Fig. 2. The fish was allowed 0.5 hr to adjust to each new arrangement; after this adjustment a second record of the time spent in the various sections of the aquarium was made. When coverts were paired, positions of the coverts were then reversed and the above procedure repeated. Generally, the black and clear coverts of each type were presented together in positions 8 and 14. The black bottom plate was presented in position 11 and only one trial for each fish was conducted. Since there was no significant difference in utilization of coverts between positions 8 and 14, the coverts in reversals of position were considered replicates. Eight fish used separately with paired coverts in the two different positions provided 16 replicates. The black bottom plate, which was not paired with a clear covert, had eight replications.

To investigate further the role of an area of darkness only, in relation to that of quiet water only, an additional set of trials was carried out using the black overhead plate with two sides and the clear overhead plate with three sides. This gave a combination not offered by any of the other choices: an area of darkness with a visual reference but no quiet water vs quiet water with no darkness nor visual reference.

The above series of trials was conducted for each fish at each of the two water velocities (12 and 28 cm/sec).

The utilization of coverts in seconds was calculated as an average of the replicates of each covert type at the same water velocity. Duncan's new multiple range test was used in comparing the differences in utilization among the various types of coverts, and Student's *t*-test for paired samples was used to make specific comparisons between two coverts or of one covert type under two different conditions.

RESULTS

Some types of coverts were virtually ignored whereas other types were utilized almost 100% of the time. Utilization of coverts was related to the environmental features offered and the water velocity.

Black coverts were highly preferred over clear coverts of the same types at both water velocities (Fig. 3). Virtually no use was made of any of the clear coverts at any time, although the fish were definitely aware of their presence. Generally, the clear coverts were explored briefly by the fish early in the observation period and thereafter ignored. The clear coverts (Table 1) offered only tactual reference and, in two cases, quiet water.

In the absence of coverts, all the fish preferred section 18. The fish spent an average of 5534 sec (77% of the test time) at low velocity and 3857 sec (53%) at high velocity swimming next to the aquarium window in this section. Excursions from the section were made, but were generally confined to the edges of the aquarium. The fish seldom ventured into the middle sections.

The addition of black coverts to the aquarium altered this behavior pattern. Although the simpler black coverts (Fig. 2F, A, B,) were used moderately, section 18 was still preferred (Table 2). As coverts containing more environmental features were offered, preference shifted to these coverts. At the high water velocity all coverts were utilized more intensively than at the low velocity and the shift in preference from section 18 to the coverts occurred sooner. At

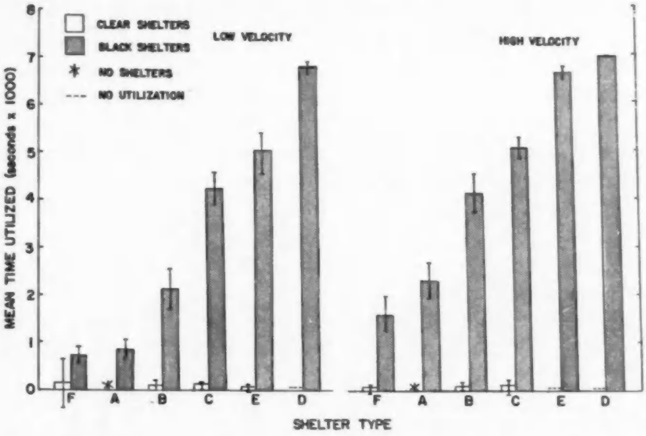

FIG. 3. Average times that the various types of shelter were utilized during 7200 sec at low and at high water velocity (12 and 28 cm/sec). The types of shelter refer to Fig. 2. Each narrow bar represents two standard errors.

the low velocity the black bottom plate was used less than section 18 (899 sec and 3276 sec), the black overhead plate with legs and section 18 were used about the same (2194 sec and 2250 sec, and the black overhead plate with two sides was preferred to section 18. Use of section 18 dropped further as the more complex coverts were introduced. At the high velocity the black bottom plate and section 18 were used about the same (2359 sec and 2168 sec), and the black overhead plate with legs was preferred to section 18.

The addition of black coverts to the previously barren experimental sections increased the use of these sections over that when no coverts were present

TABLE 1. Environmental features assumed to be supplied by the various coverts.

Type of covert	Features offered			
	Tactual reference	Visual reference	Area of darkness	Quiet water
Clear bottom plate, two sides	×			
Clear overhead plate, legs	×			
Clear overhead plate, two sides	×			
Clear overhead plate, slanted	×			×
Clear overhead plate, three sides	×			×
Black bottom plate, two sides	×	×		
Black bottom plate		×		
Black overhead plate, legs	×	×	×	
Black overhead plate, two sides	×	×	×	
Black overhead plate, slanted	×	×	×	×
Black overhead plate, three sides	×	×	×	×

TABLE 2. Average durations (in seconds) of utilization of the opaque coverts and of section 18 (the position preferred when no coverts were available).

Covert	No. of replicates	Low velocity			High velocity		
		Use of covert	Use of section 18	P	Use of covert	Use of section 18	P
Black bottom plate, two sides	16	733 ± 163[a]	2203 ± 207	<.001	1607 ± 485	2689 ± 459	<.213
Black bottom plate	8	899 ± 203	3276 ± 648	<.017	2359 ± 424	2168 ± 599	>.567
Black overhead plate, legs	16	2194 ± 456	2250 ± 487	>.557	4073 ± 436	1314 ± 359	<.002
Black overhead plate, two sides	16	4314 ± 344	1059 ± 254	<.001	5412 ± 246	633 ± 131	<.001
Black overhead plate, slanted	16	5066 ± 462	802 ± 215	<.001	6854 ± 145	34 ± 16	<.001
Black overhead plate, three sides	16	6969 ± 91	142 ± 61	<.001	7190 ± 6	<1	<.001

[a] One standard error.

TABLE 3. Average durations (in seconds) of utilization of sections 8 and 14 with and without coverts.

Covert	No. of replicates	Low velocity			High velocity		
		Use with covert	Use without covert	P	Use with covert	Use without covert	P
Black bottom plate, two sides	16	733 ± 163[a]	8 ± 5	<.004	1607 ± 485	4 ± 1	<.005
Black bottom plate	8	899 ± 203	21 ± 21	<.001	2359 ± 424	3 ± 2	<.001
Black overhead plate, legs	16	2194 ± 456	8 ± 5	<.001	4073 ± 436	4 ± 1	<.001
Black overhead plate, two sides	16	4314 ± 344	8 ± 5	<.001	5412 ± 246	4 ± 1	<.001
Black overhead plate, slanted	6	5066 ± 462	8 ± 5	<.001	6854 ± 145	4 ± 1	<.001
Black overhead plate, three sides	16	6969 ± 91	8 ± 5	<.001	7190 ± 6	4 ± 1	<.001

[a] One standard error.

(Table 3). The use of the sections increased with the presentation of the more complex coverts. At low velocity, the use of the experimental sections increased from between 8 and 21 sec without coverts to between 733 and 6969 sec with coverts. At high velocity, the use increased from between 3 and 4 sec without coverts to between 1607 and 7190 sec with coverts.

Among the black shelters, each of which offered a visual reference point, use generally increased as various environmental features were added (Fig. 4).

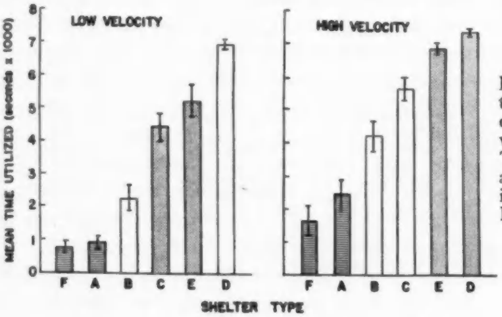

FIG. 4. Average times that various types of opaque shelters were utilized during 7200 sec at low and at high water velocity (12 and 28 cm/sec). The types of shelter refer to Fig. 2, and adjacent bars of the same shading indicate nonsignificance ($P \leq 0.05$). Each narrow bar represents two standard errors.

Addition of tactual reference points only, as in the bottom plate with two sides (Fig. 2F), did not change use ($P = 0.05$). The addition of an area of darkness (Fig. 2B, C) greatly increased utilization. Although at low velocity the bottom plate was used only an average of 899 sec (12%), the overhead plate with legs was used an average of 2194 sec (30%), and the overhead plate with two sides 4314 sec (59%). Utilization was increased with the addition of an area of quiet water, as provided in coverts E and D (5066 and 6969 sec respectively at low velocity). Although the order of utilization did not change at high velocity, there were shifts in significance between adjacent covert types. There was a shift from nonsignificant to significant difference between the overhead plate with two sides (Fig. 2C) and the overhead plate, slanted (Fig. 2E). There was also a shift from significant to nonsignificant difference between the overhead plate, slanted and the overhead plate with three sides (Fig. 2D).

Utilization of all black coverts increased at the higher velocity (Table 4). The increase was most pronounced among the simpler coverts. Use of the black

TABLE 4. Average durations (in seconds) of utilization of the opaque coverts at low and at high water velocities.

Covert	No. of replicates	Low velocity	High velocity	P
Black bottom plate, two sides	16	733 ± 163[a]	1607 ± 485	< .092
Black bottom plate	8	899 ± 203	2359 ± 424	< .035
Black overhead plate, legs	16	2194 ± 456	4073 ± 436	< .016
Black overhead plate, two sides	16	4314 ± 344	5412 ± 246	< .016
Black overhead plate, slanted	16	5066 ± 462	6854 ± 145	< .001
Black overhead plate, three sides	16	6969 ± 91	7190 ± 6	< .030

[a] One standard error.

bottom plate, for example, increased from 899 to 2359 sec, whereas use of the black overhead plate with three sides (Fig. 2D) increased only from 6969 to 7190 sec (99%).

The investigation of the roles of darkness and quiet water in the use of coverts, carried out by offering the black overhead plate with two sides and the clear overhead plate with three sides simultaneously, demonstrated a strong preference of the fish for darkness over quiet water. The durations of use (sec) of the two types of covert at the two velocities were:

Water velocity	No. of replicates	Black overhead plate, two sides	Clear overhead plate, three sides	*P*
Low	16	4781 ± 459[a]	44 ± 13	<.001
High[b]	16	4585 ± 718	1543 ± 518	<.030
High[c]	12	6301 ± 240	485 ± 216	<.001

[a] One standard error.
[b] Tests on eight fish including three that were emaciated.
[c] Tests on six healthy fish.

This preference was less pronounced at the high-velocity trials, when the original fish were used, owing to an increase in the use of the clear covert (from 44 to 1543 sec) at the high velocity. However, this increase was due to the behavior of only three of the eight fish. When this comparison was repeated with six newly captured fish, the preference for darkness was marked (6301 sec for the black covert and 485 sec for the clear covert).

DISCUSSION AND CONCLUSIONS

This study demonstrated that yearling smallmouth bass utilize shelter in flowing water. When artificial shelter in the form of plexiglass coverts was presented to the fish, utilization of the coverts was related to the complexity of features offered and to water velocity. The results confirm the reports (Hubbs and Bailey, 1938; Cleary, 1956) of the smallmouth bass as being highly shelter-oriented during daylight hours. The factors of visual reference, area of darkness, and area of quiet water, previously reported as being involved in shelter use by other species of fish (Carlisle et al., 1964; Kami, MS, 1959), were found to be important to juvenile smallmouth bass.

The opacity of a covert was an important component. Clear coverts of all types were almost totally unused, but a black covert consisting of nothing more than a flat plate on the bottom of an otherwise barren aquarium was used to a noticeable extent. We assume that a dark area either above or below the fish functioned as a visual reference point for orientation. In the series of coverts studied, the presence of a visual reference point (covert A) was the first of a series of opaque coverts that attracted fish for a marked period of time. The black plate placed on legs was used approximately twice as much as the bottom plate. Use of an overhead plate with two black sides was much higher than that of the plate on legs. The addition of sides made the area under the plate much darker, but it is presumed also to have supplied thigmotactic reference. Darkness, however, was the more important factor, as the addition of sides to the black bottom plate (covert F) did not increase its utilization. It is assumed the sides in this case added thigmotactic stimuli but not darkness.

Although Breder and Nigrelli (1938) proposed thigmotaxis as a possible factor in the use of coverts, thigmotaxis could not be demonstrated to have a role in the use of the plastic coverts. Thigmotactic reference alone did not make a covert attractive to the fish. All clear coverts offered this feature, but were not utilized to any extent. The black bottom plate with two sides which offered this feature, and the black bottom plate which did not, were not significantly different in use. This is not to imply that thigmotaxis does not enter into shelter use at all. It may be involved in a "stimulus summation" with the other environmental features of the more complex shelters. The use due to any one feature in a complex covert could not be separated from the utilization of the covert as a whole.

An area of quiet water was important in shelter use so long as an area of darkness and a visual reference point were present. Quiet water as supplied by the clear coverts was not attractive to fish, but perhaps this was because the black covert, tested simultaneously, supplied other desirable features as well as quiet water. At low water velocity, the black overhead plate, slanted, and the black overhead plate with two sides were not significantly different in use, although the slanted plate offered quiet water whereas the two-sided plate did not. However, the two-sided plate provided a better area of darkness than the slanted plate. At the higher water velocity the slanted plate was preferred over the two-sided plate and was not significantly different in use from the black overhead plate with three sides, indicating that the importance of protection from current increases with the velocity. In agreement with these findings, Kalleberg (1958) noted that juvenile trout and salmon seek more sheltered stations with increasing water velocity. Simultaneous tests of the black covert C and the clear covert D demonstrated that the smallmouth bass had a definite preference for the black covert at both high and low water velocities. Therefore, it appears that an area of quiet water is important in shelter use only so long as an area of darkness and visual reference are present.

In the last experiment the apparent decrease in preference of the black overhead plate with two sides at the high water velocity was due to the aberrant behavior of three fish. On examination, these fish were found to be emaciated. Weight losses were 5.5, 10.0, and 33.3% as compared with weight gains of 11 -33.3% for other fish. Presumably these fish were less able to withstand higher water velocities. Their loss of weight may have been due to the suppression of feeding in subordinates by dominant fish in the holding box. We have often observed this phenomenon among smallmouth bass in aquaria, and other investigators have reported similar occurrences (Stringer and Hoar, 1955; Newman, 1956). Six more fish were obtained and tested at the high water velocity. Preference for the black covert was reaffirmed.

The higher water velocity, in addition to changing the preference of fish for certain types of coverts, increased the utilization of all black coverts. Hartman (1963) observed a similar occurrence with brown trout and the observations of Kalleberg (1958) may also apply here. The use of all black coverts, not just those offering quiet water, was affected. It may be that the stress placed on the fish by the higher water velocity heightened the shelter-seeking response, which was then applied to whatever shelter type happened to be available. The increase in water velocity had no apparent effect on the use of the clear coverts.

The increased use of the black shelters at high velocity may have been due to the effect of learning. In retrospect, it might have been better to collect new fish for this part of the experiment. However, the problem of individual and sample group differences would then have arisen. We do not believe that appreciable learning took place for the following reasons. The order in which coverts were presented (Fig. 2) was not the same as the order of increasing use, and the order of increasing use was the same at low and high velocities. Coverts with few features were used only slightly, and coverts with many features were used to a large extent, irrespective of the order in which they were presented. If the fish had learned to use the coverts in the course of the experiment, the utilization of the last coverts offered should have been disproportionately higher than those of similar coverts offered earlier in the experiment. This did not occur. Since no evidence of learning could be detected within the low-velocity trials, it is unlikely that there would be any carry-over to the high-velocity trials.

Utilization was remarkably higher in the more complex coverts. For some fish, in excess of 99% of the observed time was spent under the covert. An important factor to be considered here is that no food was available in the aquarium, and hence no feeding excursions were made from the shelters.

Utilization of the coverts may have been due in part to the novel-object effect as reported by Welker and Welker (1958). This effect was noticeable when the fish was introduced into the aquarium and when the coverts were put into position. However, the 0.5-hr adjustment period was found to be sufficient to eliminate the visible effects of this reaction.

The reasons why section 18 was preferred over all other sections in the barren aquarium cannot be stated with certainty, but they are probably much the same as those involved in utilization of the artificial shelters. The water velocity in section 18, although not the lowest in the aquarium, was very near the lowest. The light intensity in this section, as in all the corners, was the lowest in the aquarium. This section also had walls along two of the sides, which may have provided thigmotactic stimuli. In addition, the side wall contained the junction of observation window and plywood wall, an obvious visual reference point. The window itself, appearing black from inside the aquarium, may also have played a part in the attractiveness of this section. A similar effect was observed by Hartman (1963). Under conditions of no shelter, the majority of fish in his stream aquarium aligned themselves along the observation window.

The features offered by some of the artificial shelters were attractive enough to the fish to cause reduction in use or even desertion of the previously favored position, as well as an increase in use of open areas of the aquarium.

ACKNOWLEDGMENTS

We are grateful to Mr Harold Breon of Linden Hall, Pennsylvania, upon whose property these studies were made.

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Lakeward Migration of Young Rainbow Trout (*Salmo gairdneri*) in the Upper Lardeau River, British Columbia

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Lakeward migration of rainbow trout fry was studied in the upper Lardeau River, where the young emerge from a spawning area immediately below the outlet of Trout Lake utilized by large trout from Kootenay Lake, about 56 km downstream. Most fry move downstream towards Kootenay Lake shortly after emergence; however, some, particularly later in the emergence period, move upstream into Trout Lake. Field observations and experiments suggest that water temperature may be important in inducing different responses to water current in these fish, but may not play such a predominant role or operate at the same levels as proposed earlier for control of young trout migration in the Loon Lake system.

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INTRODUCTION

The population of large rainbow trout in Kootenay Lake which at spawning time move about 56 km (35 miles) up an *inlet* river (the Lardeau) and spawn immediately below the *outlet* of Trout Lake has been the subject of considerable interest for a number of years (Larkin, 1951; Cartwright, 1961; Northcote, 1962). Although seasonal timing and other aspects of the adult migration have been investigated, relatively little is known of that of the young. Most of the fry emerge less than 300 m from Trout Lake but in their migration to Kootenay Lake move over 50 km downstream. Northcote (1962), arguing from observations of the migratory behavior of juvenile rainbow trout in relation to water temperature in another lake system, predicted the pattern of fry movement in the Lardeau River from its temperature characteristics. The following are pertinent to the present study: most fry emerging in late June to early July would move downstream at night towards Kootenay Lake; most fry emerging in the upper reaches after early July would not move downstream during that summer; some fry in the upper reaches in late July and August would move upstream towards or into Trout Lake during the day.

These predictions seemed reasonably consistent with data available then at Lardeau River, but the observations were limited, particularly those which would detect upstream movement of fry. During part of the summers of 1966 and 1967 it was possible to examine briefly the migratory behavior of recently emerged fry in the upper Lardeau River, and to conduct some simple field

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experiments testing the importance of water temperature in affecting direction of migration of the young trout. These are reported herein.

MATERIALS AND METHODS

Water temperature was recorded with Weksler continuous recorders at two stations near the edge of the upper Lardeau River, one near the Gerrard bridge and the other near Y-camp (Fig. 1). Water level was measured at Gerrard by a staff gauge graduated in 3-cm intervals. Light intensity was determined as described by Northcote (1962) without use of a scotopic (dark adapted human eye) filter on the photometer.

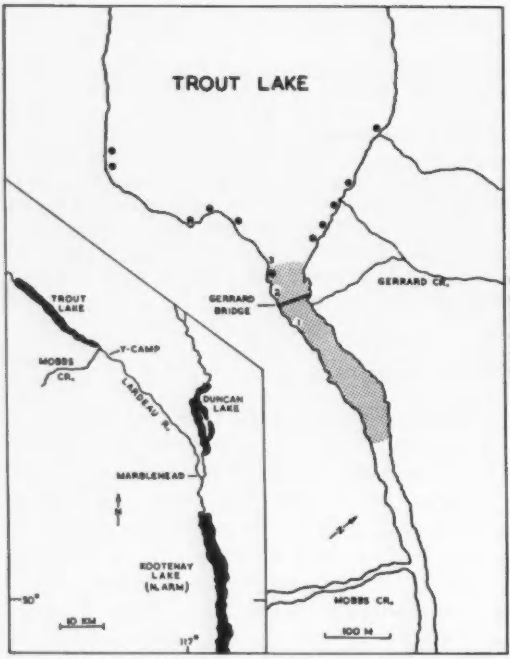

FIG. 1. The upper Lardeau River originating from Trout Lake. Solid circles refer to seining stations, numbers to location of fry traps; stippling indicates the major area for fry emergence; inset shows relation of the system to the north arm of Kootenay Lake.

Information on movement of fry, both upstream and downstream, near the edge of the river was obtained by operating traps anchored to the stream bottom at depths of about 30 cm (Fig. 2A). These traps (150 × 90 × 30 cm deep) were covered with 2.5-mm mesh galvanized hardware cloth and were divided by a screen partition into two halves, each fitted with a V-type screen lead. Leads were removable to permit recovery of the catch and cleaning. Traps were located along the south bank of the river at three stations in 1966 and at one station (3) in 1967 (Fig. 1). All the traps were checked twice daily, early in the morning and again at dusk. Fry in both sections were counted, measured, and usually released.

To sample fry moving downstream further away from the river edge, two sizes of fine-mesh fyke net were used. The smaller nets (30 cm^2) were operated during periods of intensive downstream fry movement, whereas larger nets (30 × 60 cm) were used when few fry were moving in August. These nets were operated for 24-hr periods near the Gerrard bridge and at Y-camp, about 1350 m downstream from the bridge (Fig. 1).

Observations on areas occupied by trout fry during day, dusk, and dawn periods were made within 1-m squares along the river edge using 7 × 50 binoculars during the day and, at light intensities below about 10 lux, a small flashlight. Squares were marked off by means of string above the water surface (Fig. 2A) or heavy wire resting on the bottom and were divided into halves (onshore and offshore).

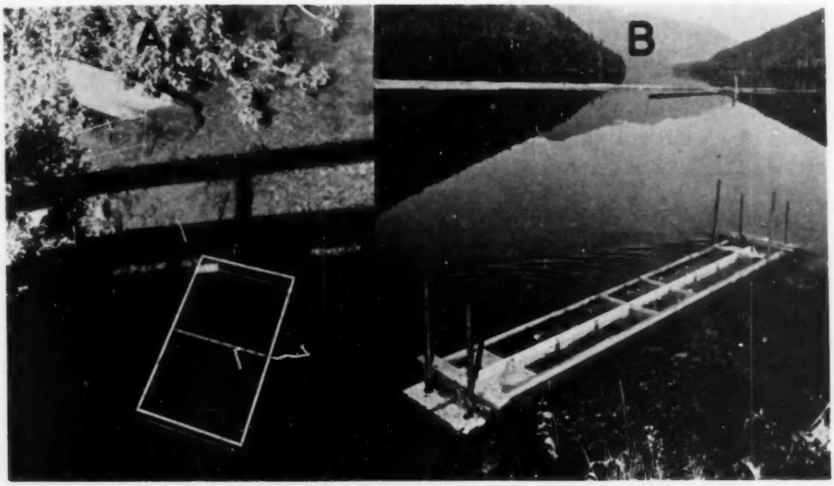

Fig. 2. (A) Trap used to record upstream and downstream movements of rainbow trout fry; 1-m square counting area at river edge above trap; (B) experimental flume used to study movement of rainbow trout fry in location at outlet of Trout Lake.

Data on distribution and abundance of fry near the head of the river and in Trout Lake itself were obtained by making midday and midnight hauls with fine mesh seines, 2 × 3 m, and 2 × 9 m, at a series of stations (Fig. 1). Additional observations were made from the bridge and shore as well as under water with diving gear.

Experiments on movement of fry were conducted in a wooden flume, 4.3 m long, divided into two equal channels by a central partition (Fig. 2B). Each channel (30 cm wide) was subdivided into nine sections, a middle "holding" section 60 cm long, six alternate "pool" and "riffle" sections 30 cm long (three upstream and three downstream from the holding section), and a trap section at each end. Baffles between sections were adjusted to simulate pool and riffle conditions. The bottom, except for the trapping sections, was covered with gravel and small stones to provide cover and resting areas. Subsurface flow (6-cm depth) at the head of each channel averaged between 10 and 12 cm/sec. Water depth was kept at 13 cm. In most experiments (1966, 1967) fry were introduced into the holding sections in the morning and retained there throughout the day by screen barriers at each end. These were removed at dusk, giving the fry access to pool or riffle sections either upstream or downstream and into the traps at the upper or lower end of the channels. In a few experiments (1967), fry were placed in holding sections at dawn, and released by midmorning, and distribution was recorded before dusk. Movement was expressed as the percentage of fry that moved into traps at the upper or lower ends of each channel. Groups of 50 fry (1966 experiments) and 32 fry (1967 experiments) were used in each channel. Fry in 1966 experiments ranged between 26 and 36 mm (average 30.3) whereas those in 1967 were slightly larger (29–47 mm, average 34.6). Screen covers over both channels prevented predation or escape of fry.

CHARACTERISTICS OF THE UPPER LARDEAU RIVER

Water temperature, level, and discharge data for the Lardeau River are given by Cartwright (1961). Maximum discharge of the river usually occurs in June and may exceed 140 m³/sec. Thus both discharge and water level are declining while water temperature is increasing during most of the emergence period (Fig. 3). At high water level the river at Gerrard floods back into marginal vegetation and debris, particularly along its south bank, so that recently emerged

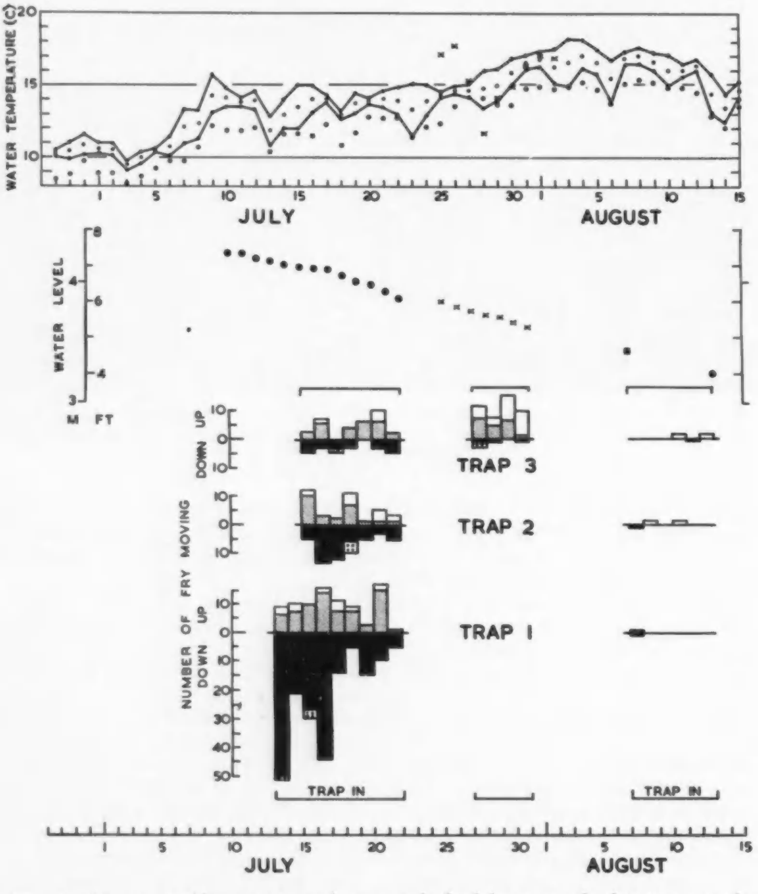

FIG. 3. Movement (downstream and upstream) of rainbow trout fry into traps on the upper Lardeau River in 1966 (July 13–22; August 7–12) and 1967 (July 27–30). Solid dots show 1966 maximum, mean, and minimum water temperatures at Gerrard; open circles, means at Y-camp. Crosses show mean water temperatures and levels at Gerrard during late July 1967. Solid and hatched bars represent night movement; open and dotted bars represent day movement. Traps 1 and 2 not operated in the late July period, 1967.

fry may inhabit, temporarily at least, areas where temperature and flow conditions are different from those in the river proper. During July there are marked and rapid daily changes in temperature of the river at Gerrard (Fig. 3), probably a result of wind action on Trout Lake. The lake is stratified thermally throughout summer so that internal seiches could readily produce such fluctuations at the outlet.

Since, throughout the summer, most tributary streams are appreciably cooler than the river at Gerrard, Lardeau River temperature decreases downstream. Although a slight decline in temperature (usually less than 1 degree C) may occur between Trout Lake outlet and the entrance of Mobbs Creek, it is colder water from this creek that is largely responsible for the consistently lower temperature recorded at Y-camp (Fig. 3).

RESULTS

EMERGENCE AND DISTRIBUTION OF FRY

The majority of spawning in the upper Lardeau River occurs within a 300-m section below Trout Lake (Fig. 1), with a few redds located as far downstream as the mouth of Mobbs Creek; redds are not confined to either marginal or central sections of the spawning area (G. F. Hartman, personal communication). In 1966 emergence began after maximum water level was reached, probably early in July. No fry were evident on June 25 but small groups were seen along the south bank of the spawning area on July 9. Recently emerged fry (indicated by incompletely resorbed yolk sacs) were present throughout July and some were taken as late as August 10 in 1966.

The diel timing of final exit from the redds was not determined, but recently emerged fry appear near the river margins of the spawning area where small groups may be seen holding position in the current during the day, or moving about in quiet water, usually not more than a meter away from the margin. Although sections of the river away from the edge were searched with binoculars, fry were rarely seen there during the day. Surface water velocities were in the order of 1 m/sec in most places away from the river bank, but some eddies and lower velocity areas were present. Recently emerged fry were rarely seen there.

Distribution and abundance of recently emerged fry were recorded in a 1-m square area along the south bank of the river immediately above Gerrard bridge (Fig. 2A). From late afternoon until dusk total numbers increased in the area, particularly as light intensity began to decline sharply (Fig. 4, upper right). Because fry were rarely seen offshore beyond the counting area during the day, the increase in numbers at evening may be due in part to emergence and exit of fry from offshore redds at that time. A shift in distribution of fry within the 1-m area also took place in the evening (Fig. 4, upper left). Numbers in the offshore half initially increased and then decreased whereas those in the onshore half increased. Since *total* numbers increased at dusk as well, some fry must have come from regions well beyond the 1-m square observation area,

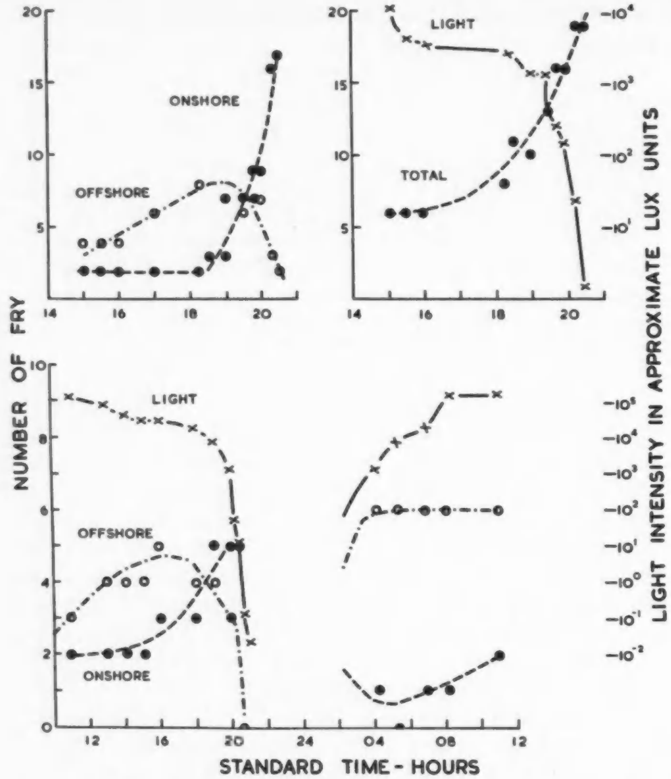

FIG. 4. Changes in number and distribution of trout fry within 1-m square areas along the edge of the upper Lardeau River. *Upper*, recently emerged fry along south bank, July 14, 1966; lower, older fry along south bank, July 27–28, 1967. "Onshore" refers to 0.5-m strip along river edge;"off shore" to adjoining half away from edge; "total" to entire square meter.

possibly emergents from offshore redds, since access to the counting area from edge regions immediately upstream was restricted (Fig. 2A).

Older fry also shifted distribution as evening approached in a manner similar to that shown by younger individuals (Fig. 4, lower). The following morning they had returned to the typical day distribution within the observation area. Similar changes were recorded at several locations along the river edge on successive days.

DOWNSTREAM MOVEMENT

All three traps took fry moving downstream (Fig. 3), in numbers which usually decreased from the lowermost (trap 1) to the uppermost (trap 3) location. Most likely this was a reflection of redd distribution and emergence pattern, as the area near the south bank immediately below the bridge was heavily utilized by spawners (G. F. Hartman, personal communication). Downstream movement occurred largely overnight. It gradually decreased after mid-July, and virtually ceased by early August in 1966. Very few fry moved downstream at the trap 3 site during late July in 1967, a period which was not sampled the previous year.

The diel pattern of downstream movement was recorded at two locations on the upper river, one about 4 m off the south bank near the Gerrard bridge and the other at Y-camp (Fig. 5). Fry exhibited maximum rates of downstream movement shortly after dusk, with reduced movement continuing throughout the night, a pattern typical for downstream movement of recently emerged fry (Northcote, 1962). Although very few fry were moving downstream near the center of the river at the upper end of the emergence area, appreciable numbers were taken near the center of the channel at Y-camp further downstream. This would suggest that the movement was not restricted to short distances near the site of emergence but represented a general downstream migration of young fish.

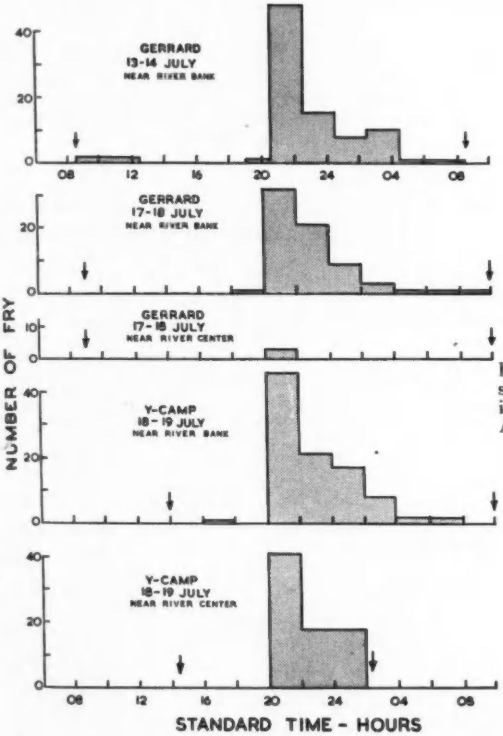

FIG. 5. Diel periodicity of downstream movement of rainbow trout fry in the upper Lardeau River, 1966. Arrows indicate start and end of sampling period.

Most of the fry moving downstream ranged between 25 and 35 mm long (Fig. 6) and vestiges of yolk material were still evident in many. Fry taken at Y-camp were slightly larger than those at Gerrard and were comprised of fewer individuals still retaining evidence of yolk material.

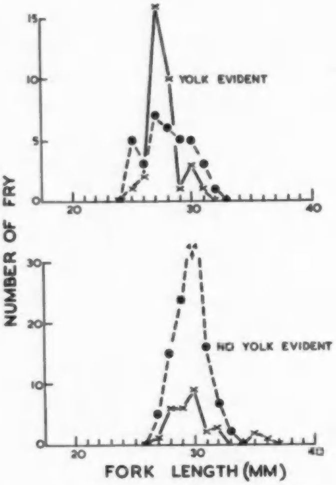

FIG. 6. Length–frequency distribution of rainbow trout fry moving downstream at Gerrard (*upper*, July 17–18) and Y-camp (*lower*, July 18–19) in the upper Lardeau River.

UPSTREAM MOVEMENT

Trout fry also moved upstream into traps along the upper Lardeau River and on some days, particularly during mid- to late July, upstream movement considerably exceeded downstream movement (Fig. 3). In contrast to the pattern of downstream movement, many of the fry moving upstream did so during the day (Fig. 3). Fry moving upstream tended to be larger than those moving downstream into the traps (Fig. 7).

At the start of the emergence period early in July, fry were never seen far above the Gerrard bridge and the greatest concentrations were evident immediately downstream from the bridge, particularly along the south bank of the river. Throughout July and early August regular checks and seine hauls for fry were made along both sides of the river and along the lakeshore near the river outlet. Results indicate a gradual upstream spread of the rainbow fry and a movement along both shores of the lake (Fig. 8), well beyond the furthest upstream redd. Although no fry were taken at the four standard seining stations furthest up the shores of Trout Lake (Fig. 1) in July, several were taken at each of these locations in early August, again indicating that some young trout do move upstream into Trout Lake and at least a short distance along its shores. These fry ranged from 28 to 48 mm long and included a few individuals still possessing remnants of yolk.

Fry taken from seining stations near the outlet end of Trout Lake were almost certainly progeny from Lardeau River spawners. No trout fry were

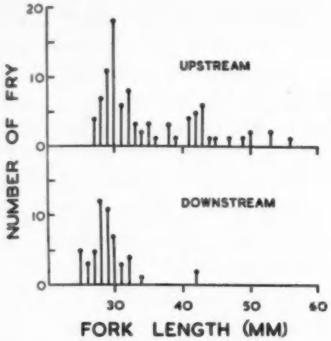

FIG. 7. Size of fry moving upstream and downstream into traps on the upper Lardeau River, mid- to late July, 1966 and 1967.

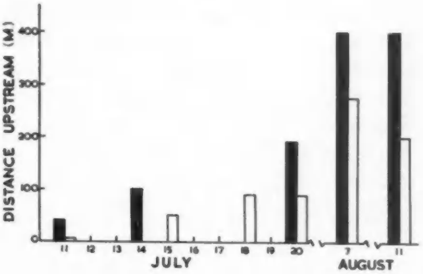

FIG. 8. Furthest distance upstream from Gerrard bridge that rainbow trout fry were observed or captured along shores of upper Lardeau River and Trout Lake in 1966 (solid bars "south" shore, open bars "north" shore; see Fig. 1 for locations).

seen during attempts to collect them from other small streams entering Trout Lake up to 7 km away from the outlet in August 1966.

SUMMARY OF FRY MOVEMENTS IN UPPER LARDEAU RIVER

The following pattern of young trout movement from the Gerrard emergence area is suggested:

(1) Most young leaving redds during early and middle phases of emergence (usually late June to mid-July) maintain position near the river margin during the day and concentrate at the very edge towards dusk.

(2) Many of these recently emerged fry as well as those emerging at night are unable to maintain position in the river during darkness and move downstream towards Kootenay Lake; in the emergence area fry movement is concentrated near the river edge, but further downstream (ca. 1 km) occurs more generally across the river.

(3) Older fry as well as some recently emerged fry move upstream along the river margin towards and into Trout Lake particularly near the end of the emergence period (mid- to late July); such movement occurs during daylight, and also to some extent in darkness.

(4) Some of the fry entering Trout Lake from the Lardeau River may leave that lake when older and move downstream towards Kootenay Lake.

FLUME EXPERIMENTS

Overnight tests were made on the response to water current of young rainbow trout from the upper Lardeau River when placed in the experimental flume (Fig. 2B) supplied first with warm water draining off the surface of Trout Lake and later with cool water from Gerrard Creek (Fig. 1). Although downstream movement occurred at both temperature ranges tested, there appeared to be appreciably less in experiments with warm Lardeau River water than in those with the cool water from Gerrard Creek (Fig. 9). On the other hand upstream movement was greater in warm water than in cool water. Replicate experiments in the two channels gave reasonably consistent results as did those between the two successive years. The one set of experiments where fry moved during the day gave results similar to those for the more extensive night series (Fig. 9).

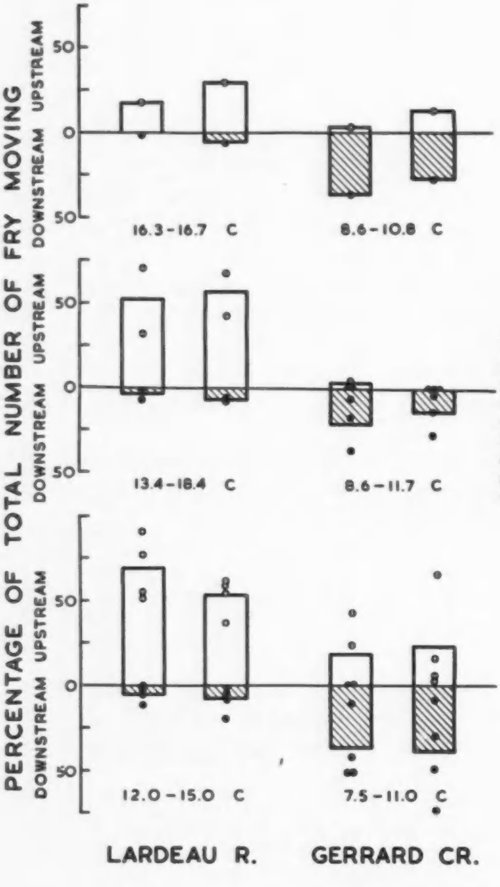

FIG. 9. Differences in movement of rainbow trout fry in replicate experimental flume channels supplied with warm (Lardeau River) and cool (Gerrard Creek) water. Open and closed circles give percent movement for each experiment, bars represent averages. *Upper*: day experiment, 1967; *middle and lower*: night experiments 1967 and 1966, respectively.

DISCUSSION

The 1966 observations on migratory behavior of rainbow trout fry in the upper Lardeau River suggest that the majority of young fish move down the river at night, shortly after emergence from their redds. This movement occurs throughout the upper reaches of the river and apparently is continued further down the system so that many of the young probably reach Kootenay

Lake within a few weeks after emergence. Dr A. H. Acara has provided information on downstream movement of rainbow trout fry from operation of sampling gear at Marblehead (Fig. 1) near the confluence of the Lardeau and Duncan rivers. In 1966, downstream movement of the fry there extended from July 6 to August 31, the majority moving between July 10 and July 25. Virtually all fry moving downstream did so at night. The size of the fry increased during the period of migration — early migrants were between 24 and 30 mm (probably recently emerged, see Fig. 6), whereas those moving late in July and in August ranged from 26 to 54 mm. Some of the larger fry must have spent several weeks either in the middle reaches of Lardeau River or possibly in Trout Lake before moving downstream.

Throughout most of the emergence period early in July, water temperature of the Lardeau River at Gerrard was usually below that at which appreciable upstream movement would be expected if the current–temperature response of the fry was similar to that of Loon Lake young (Northcote, 1962). At water temperatures appreciably below 14 C, large numbers of trout fry moved downstream in the Loon Lake streams (inlet, Hihium Creek, and even in its outlet). Certainly if low temperature is important in inducing downstream movement of young Lardeau fry, then this response should be enhanced the further they progress, since the river, fed by glacial streams, becomes appreciably cooler as well as more turbid in its lower regions (Cartwright, 1961). The daily duration of downstream movement also should be increased as turbidity increases.

Other features of the downstream movement of the Gerrard stock rainbow fry were similar to that in the Loon Lake system. In both there was a concentration of fry at the stream margin with decreasing light intensity in the evening prior to the onset of downstream movement. Likewise the sharply peaked periodicity of this movement after dusk was characteristic of both systems.

Although some trout fry move upstream towards Trout Lake at water temperatures below that characteristic of such behavior in the Loon system, at the uppermost trap site near the mouth of the lake, upstream movement was highest in late July when average water temperatures usually exceeded 15 C (Fig. 3).

However, there were several differences in behavior of the young trout between the Loon and Lardeau River systems. In the latter some upstream movement, although limited, did occur at least for short distances in July when daily mean water temperatures were below 14 C. Also many of the fry moving upstream into traps, or taken in Trout Lake itself near the outlet, were below the size (40 mm) at which Loon fry commonly commence upstream movement. Furthermore results of experiments conducted in the flume with young Lardeau River trout fry were not entirely consistent with earlier experimental studies on Loon Lake trout fry (Northcote, 1962, Fig. 10). Although in both instances downstream movement was greater at lower water temperatures, a considerable amount of upstream movement was exhibited by the recently emerged Lardeau fry (especially in 1966 experiments) at water temperatures somewhat lower than those usually associated with such movements in older and larger Loon Lake trout fry. Nevertheless the field observations and experiments both suggest that water temperature may be an important factor regulating direction of movement of young trout in the Lardeau River system.

The fact that some young trout, apparently originating from Kootenay Lake parents, after emergence from redds in the upper Lardeau River move upstream and enter Trout Lake is of considerable interest. That these young might be offspring of outlet spawners from Trout Lake cannot be ruled out. However, a fish weir was operated across the Lardeau River at Gerrard from 1915 to 1949 to capture rainbow trout spawners for fish cultural purposes. Observations here indicated that few if any rainbow trout from Trout Lake ever utilized its outlet for spawning. If, on the other hand temperature conditions in the upper Lardeau River are important in regulating direction of trout fry migration, then the population of Kootenay Lake trout spawning at the outlet of Trout Lake may have developed a temporally adjusted spawning period so that the majority of young usually emerge at temperatures conducive to their downstream movement and entry into Kootenay Lake. Most young emerging from late spawning fractions of the population on years such as 1967, when Lardeau River temperatures were slightly higher than those in mid-July of 1966, may well enter Trout Lake rather than Kootenay Lake. Assuming that reproductive homing would be highly developed, the apparent lack of adult trout from Trout Lake spawning in its outlet suggests that the young trout fry which enter Trout Lake from the upper Lardeau River do not necessarily remain there, and many may move downstream again towards or into Kootenay Lake at a later stage. The sudden appearance of small schools of juvenile rainbow trout (yearling and older) at the outlet of Trout Lake in August 1966 may have been representative of such downstream migration.

The present observations on migratory behavior of young rainbow trout in the upper Lardeau River indicate that some fry do move upstream into Trout Lake. Although the timing of such movement does not seem to be entirely consistent with the temperature levels which induce different current response of young trout in the Loon Lake system, water temperature is probably an important factor. Obviously further study of migratory behavior of the young trout is required, both to clarify the factors controlling direction of movement and to determine the extent and significance of movement of Kootenay Lake stock to Trout Lake, a matter of some importance to management of this valuable population.

ACKNOWLEDGMENTS

Stimulating discussions with S. B. Smith, former Chief Fisheries Biologist of the Fish and Wildlife Branch (now Director of Fish and Wildlife Division, Department of Lands and Forests, Alberta), prompted me to make the observations reported herein. J. F. Maher and A. D. Maurer willingly spent long hours assisting in observations and collections under trying field conditions. H. Kniess and Dr A. H. Acara arranged construction of fry traps and the experimental flume; the latter also provided information on young trout migration in the lower Lardeau River. Drs G. F. Hartman, J. D. McPhail, T. Lindström, and Mr E. H. Vernon gave valuable suggestions and criticisms of the manuscript. The study was supported in part by a grant from the National Research Council of Canada.

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In vitro Degradation of DDT by Intestinal Contents of Atlantic Salmon (*Salmo salar*)

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CHERRINGTON, A. D., U. PAIM, AND O. T. PAGE. 1969. *In vitro degradation of DDT by intestinal contents of Atlantic salmon (Salmo salar).* J. Fish. Res. Bd. Canada 26: 47–54.

Fresh mid- and hind-gut contents of young, hatchery-reared Atlantic salmon degraded *p,p'*-DDT to *p,p'*-TDE in vitro. Most of the ability to degrade DDT, attributable to intestinal microflora, was eliminated by streptomycin and by heat-sterilization of the gut contents. Since TDE is less toxic than DDT, the activity of intestinal microorganisms may increase the chances of survival of fish that ingest DDT-contaminated prey.

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INTRODUCTION

DEGRADATION OF DDT has been demonstrated in only three species of fish: cutthroat trout (Allison et al., 1963, 1964), Atlantic salmon (Premdas and Anderson, 1963; Greer and Paim, 1968), and rainbow trout (Wedemeyer, 1968).

At present little conclusive information is available concerning the exact sites and mechanisms of DDT breakdown in fish. The suggested degradation of DDT in fish livers (Klein et al., 1964) has been confirmed recently through experiments with rainbow trout liver homogenates that converted DDT to DDE (Wedemeyer, 1968). Wedemeyer also proposed that ingested DDT may be degraded to DDE and TDE by the intestinal microflora of fish. However, he did not analyze the intestinal contents or excreta for DDT derivatives. Thus the suggested participation of fish intestinal contents in DDT degradation remains uncertain.

Accordingly, the present study was undertaken to elucidate the conversion of DDT by the alimentary tract contents of Atlantic salmon in vitro.

MATERIALS AND METHODS

The salmon for this study, reared at the South Esk Fish Culture Station, N.B., were approximately 2 years old, 16–20 cm in fork length, and weighed 31–60 g. In the laboratory, they were kept in flowing water at about 10 C and were fed finely ground beef liver, of which they consumed about 0.2 g per gram of salmon daily.

Digestive tract contents for experiments were obtained from freshly killed salmon by opening the body cavity and massaging the contents of the mid- and hind-guts onto a watchglass. Each salmon yielded about 5 mg of gut content for every gram of salmon, i.e., about 150–300 mg for each sample.

In the first three experiments, samples of the intestinal material were made up by placing four aliquots of the gut contents of each fish in a 6-ml test tube, and combining the fifth aliquot with single aliquots from the other fish for a composite sample. In the remaining two

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experiments, the gut contents from all fish were pooled and mixed before being divided equally between the desired number of test tubes. To promote the rapid microbial growth found necessary for microbial degradation of DDT (Chacko et al., 1966), 0.5 ml of sterile nutrient broth (Difco Laboratories) was mixed with each sample. In the first experiment, 1, 2, 4, or 8 mg of 99.9% *p,p'* isomer of DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane) (Geigy Chemical Corp.) were added to the desired number of samples. In the subsequent experiments, the amount of DDT added per sample was 4 mg. A control, consisting of 4 mg of DDT in 0.5 ml of nutrient broth, was usually appended to each set of samples. The sample tubes were plugged with cotton and their contents were mixed vigorously for 5 min on a vortex mixer.

For two experiments with sterile intestinal contents, each tube containing the gut content-broth mixture was autoclaved at 121 C. Instead of adding DDT to the sterilized sample, the latter was transferred aseptically to another test tube that contained DDT, autoclaved previously for 60 min at 121 C. This procedure was possible because heat-sterilization caused no degradation of the DDT, although less pure, technical preparations of DDT may decompose at temperatures as low as 100 C (Fleck and Haller, 1946; Metcalf, 1955). Sterility tests were done either in nutrient agar (Difco Laboratories) plates or in nutrient broth, incubated for 72 hr at 37 C.

All samples were incubated without shaking, either anaerobically in jars purged with nitrogen and containing a layer of Oxsorbent (Burrell Corp.), or aerobically. The incubation temperature was usually 37 C and incubation period usually 72 hr.

The addition of buffers to the samples was deemed unnecessary because the *p*H values of the samples remained remarkably stable. Thus, the *p*H of freshly obtained intestinal contents, approximating the values given in literature for several species of fish (Barrington, 1957), ranged between 6.5 and 6.8, that of the broth was about 6.8, and that of the final mixture was 6.5–6.8. During incubation the mixtures usually became slightly more acid, but always by less than 0.5 *p*H units.

Immediately after incubation, 1 ml of *n*-hexane (Fisher Scientific Co.) was added to each sample for the extraction of DDT residues, and the sample was shaken for 5 min on a vortex mixer. Upon standing for 15 min, the hexane separated from the sample and cleared. Further cleanup of the extract was unnecessary since the chromatographic separation of DDT and its metabolites was complete and free from interference by other compounds in the extract.

Thin-layer chromatographic analysis of the extracted residues was done on sheets coated with aluminium oxide and polyvinyl alcohol (Eastman Chromagram 6062). Prior to use, the sheets were activated at 90 C for 30 min. After the application of 20-μliter spots of the extracts at the line of origin, each sheet was stood in *n,n'*-dimethyl formamide that was allowed to migrate to the origin. The separation of DDT and its metabolites was done in an Eastman Chromagram Developing apparatus, containing a solvent mixture that consisted of 4% (v/v) of heavy paraffin oil (Fisher Scientific Co.) in *n*-hexane. The solvent front rose at the rate of about 1 cm in 10 min at room temperature. As was established with a standard solution of the *p,p'*-isomers of DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene) (Geigy Chemical Corp.), DDT, and TDE (DDD; 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane) (Rohm and Haas Co.) (1.0 mg of each per ml of *n*-hexane), the respective R_f values were about 0.71, 0.51, and 0.29. A 20-μliter spot of the standard solution was run on every chromatogram. Developed chromatograms were viewed under short-wave ultraviolet (ca. 250 mμ), and tracings were made of all visible spots.

EXPERIMENTS

DEGRADATION OF DDT AT 37 C

The first experiment was designed to test the ability of salmon gut contents to degrade DDT in vitro. As it was not known whether oxygen could alter the activity of the gut contents, a part of the experiment was done anaerobically.

Gut contents from 48 salmon were used in making up 12 sets of sample tubes, each set comprising five tubes. DDT was added to the first four samples in each set; the fifth sample was kept free from DDT. All samples and controls were incubated at 37 C, six sets in air and six in an anaerobic environment. Two sets from each environment were analyzed after 12 hr, another two after 36 hr, and the last two after 72 hr.

To determine whether the amount of DDT in any of the samples had been lethal to the intestinal microflora, a loopful of each incubated sample was transferred into a separate tube of sterile nutrient broth. Since bacterial growth developed in every tube, none of the concentrations of DDT appeared to have acted as an antiseptic.

Chromatography revealed spots of DDT in the extracts of the controls and of those samples in every set to which DDT had been added initially (Fig. 1–3, C_1, G_1–G_8). No DDT was detected in the extracts of the DDT-free samples (Fig. 1–3, C_2); thus, the salmon gut contents did not contain measurable concentrations of DDT before the experiment.

After 12 hr of anaerobic incubation, very small spots of TDE developed from those gut content samples to which either 4 or 8 mg of DDT had been added (Fig. 1, G_4, G_8). No TDE was detectable in any samples incubated aerobically for 12 hr. TDE appeared in every DDT-treated sample after 36 hr of incubation, but the areas of the TDE spots from the anaerobic samples were distinctly larger than the areas of the corresponding spots from the aerobic samples. However, little difference remained between the spot sizes from samples incubated anaerobically and aerobically for 72 hr (Fig. 2–3).

That the TDE was produced through in vitro degradation of DDT and that salmon gut contents were required for the degradation was inferred from the failure to extract demonstrable amounts of TDE from the DDT-free samples and from the controls containing DDT in broth but lacking gut contents (Fig. 2–3, C_1, C_2). No trace of DDE was discovered in any of the samples.

Degradation of DDT at 10 C

As was just shown, the degradation of DDT took place at 37 C, i.e., at a temperature much above that to which the salmon and their microflora had been acclimated. Therefore, to determine whether DDT could be degraded in salmon gut contents at the acclimation temperature, the procedure was repeated at 10 C. Three sets of five gut-content samples were prepared, each set from four salmon. DDT was added to four samples in each set. All samples and controls were incubated aerobically for 72 hr, two sets at 10 C and one, maintained as a control set, at 37 C.

The results obtained at 37 C paralleled those of the first experiment, TDE appearing in every DDT-treated sample. All DDT-treated samples produced TDE at 10 C as well but, judging from the relatively small spot areas (Fig. 4), at a markedly lower rate than at 37 C. Again, no TDE was found in any of the DDT-free samples, TDE was the sole visible degradation product of DDT, and bacterial growth developed in broth cultures that were inoculated from the samples that had been kept at 10 C.

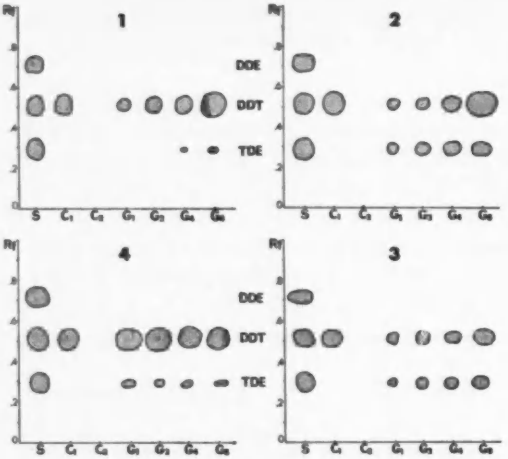

FIG. 1–4. Tracings of chromatograms of extracts from salmon intestinal contents to which p,p'-DDT was added: (1) incubated anaerobically for 12 hr at 37 C; (2) incubated anaerobically for 72 hr at 37 C; (3) incubated aerobically for 72 hr at 37 C; (4) incubated aerobically for 72 hr at 10 C. S = standard solution (p,p'-isomers of DDT, DDE, and TDE); C_1 = control: DDT in nutrient broth; C_2 = samples lacking DDT; G = samples containing DDT (each subscript denotes the amount of DDT (mg) per sample).

EFFECT OF STREPTOMYCIN

Although the first two experiments showed that DDT in salmon gut contents was degraded readily, the role of salmon intestinal microflora in the degradation remained indefinite. The purpose of the remaining experiments was to explore more directly the requirement of the microflora in the degradation.

The next experiment consisted of a comparison of the DDT-degrading abilities of salmon gut contents in the presence and in the absence of a high concentration of streptomycin sulfate, used to inhibit the activity of the microflora. Gut contents from 10 salmon were divided to make up two sets of six samples. DDT was added to five samples in each set; 800 μg of streptomycin were added to three of the samples containing DDT and to the sample lacking DDT. A control in each set contained DDT and streptomycin in broth. Both sets were incubated aerobically.

Chromatography of hexane extracts from every DDT-containing sample revealed distinct spots of TDE in addition to DDT, but the amounts of TDE were distinctly smaller in those samples that had been treated with the antibiotic (Fig. 5, A). Neither of the controls yielded degradation products of DDT. Thus, the rate of degradation was lowered markedly in the presence of streptomycin. That degradation was not eliminated was possibly due to continued activity of some species among the microflora that were unsusceptible to streptomycin.

EFFECT OF HEATING

To reduce microbial activity in the gut contents more effectively, sterilization by autoclaving was substituted for the treatment with streptomycin. Gut contents from 12 salmon were pooled and aliquots of the mixture were divided among 24 test tubes. DDT was added immediately to 12 of the samples, whereas the remaining 12 samples were autoclaved for 60 min before being mixed with sterile DDT. Two of the four controls, consisting of DDT in nutrient broth, also were prepared aseptically. All samples were incubated aerobically.

Degradation of DDT to TDE occurred in all unsterilized samples (Fig. 6, G). In contrast, only traces of TDE were extractable from 11 of the heated samples and none from one sample (Fig. 6, H). Extracts of the controls yielded spots of DDT alone. All broth cultures, inoculated with material from the heated samples, remained sterile.

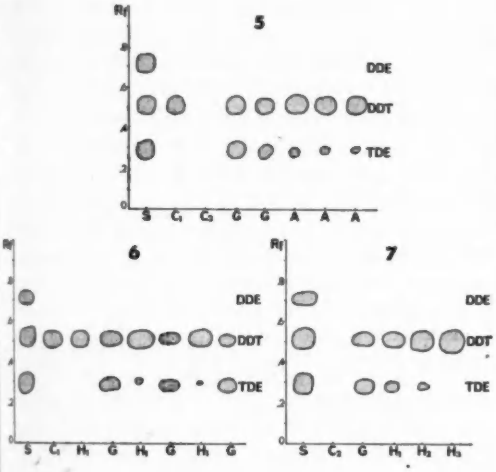

FIG. 5–7. Tracings of chromatograms of extracts from salmon intestinal contents to which p,p'-DDT was added: (5) treated with streptomycin; (6) heated in autoclave for 60 min; (7) heated in autoclave for 15 min at a time. S = standard solution (p,p'-isomers of DDT, DDE, and TDE); C_1 = control: DDT in nutrient broth; C_2 = samples lacking DDT; G = samples containing DDT; A = samples treated with streptomycin; H = heated samples (each subscript denotes the number of heat applications).

Clearly, sterilization altered the intestinal material sufficiently to render it nearly incapable of DDT degradation. However, as a few spores of the microflora might have survived the single application of heat, there remained the possibility that the residual degradation of DDT in the apparently sterile samples was attributable to microbial action.

EFFECT OF REPEATED HEATING

A further experiment was done to determine whether the degradation of DDT in salmon gut contents could be stopped by repeated sterilization. Gut contents from 20 salmon were pooled, mixed, and divided equally among 25 test tubes. DDT was added immediately to the samples in five tubes. The second set of five samples was autoclaved for 15 min prior to the addition of sterile DDT. The third and fourth five-sample sets, kept at 37 C, were autoclaved for 15 min at intervals of 24 hr, two and three times respectively. DDT was added after the final autoclaving. No DDT was added to the remaining five samples. All samples were incubated aerobically. The sterility of each sample was tested on nutrient agar.

Chromatography of the extracts of all but the fourth set of samples showed spots of TDE in addition to DDT (Fig. 7). Again, DDE was found in none of the samples. Since the areas under the TDE spots were decreased with each autoclaving, the rate of DDT degradation was evidently lowered whenever the samples were heated. However, definite bacterial growth on nutrient agar was produced only from the samples that were not autoclaved.

DISCUSSION

Our finding that DDT in the intestinal contents of Atlantic salmon was degraded to TDE rather than to DDE is highly characteristic of microbial metabolism of DDT. Only twice has DDE been found as an in vitro microbial metabolite of DDT and, then, in relatively small amounts among conspicuous amounts of TDE (Stenersen, 1965; Guenzi and Beard, 1967). The observation that the conversion of DDT to TDE in salmon gut contents proceeded in the presence of air need not be taken as a firm indication of the involvement of aerobic microorganisms in the conversion. Instead, the unshaken samples may have become somewhat deficient of oxygen, permitting the metabolism of DDT by obligate and facultative anaerobes. This possibility seems especially tenable because completely aerobic conditions have been shown either to decelerate the degradation of DDT by bacteria (Wedemeyer, 1966) or to stop the process (Stenersen, 1965; Johnson et al., 1967).

Though the presence of microorganisms seems to be the main requirement for in vitro degradation of DDT by the intestinal contents of salmon, the participation of other factors in the degradation cannot be ruled out. Thus, enzymes secreted by salmon digestive glands might have had a minor role in catalyzing the degradation in the unheated gut contents. Some nonenzymatic catalysts, especially porphyrins, are even more likely to have shared in the degradation before as well as after the autoclaving of the samples. The existence of active porphyrins in salmon intestines during the present investigation appears particularly likely because the salmon were maintained on a porphyrin-rich diet of raw liver, some of which may not have been completely digested before the intestinal contents were drawn for testing. The ability of iron porphyrins to degrade DDT has been seen repeatedly (Castro, 1964; Miskus et al., 1965; Wedemeyer, 1966; Ecobichon and Saschenbrecker, 1967), and it has been suggested that porphyrins in the livers of birds and rats (Bunyan et al., 1966) and of fish (Wedemeyer, 1968) are involved in the degradation of DDT.

Although both TDE and DDE have appeared in vivo in the blood and livers of rainbow trout that were administered DDT by mouth (Wedemeyer, 1968), our failure to discover any conversion of DDT to DDE in salmon gut contents in vitro, suggests that the metabolism of DDT to DDE in fish occurs in sites other than the lumen of the digestive tract. This suggestion is supported by the absence of DDE from the feces of rats that ingested DDT (Mendel and Walton, 1966).

Easily noticeable amounts of DDT became degraded in salmon gut contents, even if crystals of DDT were plainly visible. Because of the very low solubility of DDT in aqueous media (Bowman et al., 1960), most of its degradation was likely confined to the small part of the medium in immediate contact with the surfaces of the crystals. Since finer suspensions of DDT that expose relatively large surface areas to the suspending medium might become degraded more rapidly, our results probably do not give a true indication of the possible maximum rate of degradation.

The degradation of DDT by fish gut contents may be important in increasing the chances of fish survival in DDT-treated watersheds. As the toxicity of TDE to animals is markedly lower than that of DDT (Metcalf, 1955; Kenaga, 1966), fish that convert a significant proportion of ingested DDT to TDE before the toxicant is absorbed from the digestive tract may survive after taking in potentially lethal doses of DDT. Since the digestion of prey by fish is a slow process, usually requiring several days (Barrington, 1957), the liberation of DDT from contaminated foods is probably gradual and conducive to degradation in situ. Degradation of DDT within the digestive tract may be enhanced further by the almost immediate proliferation of the intestinal microflora that is known to follow the intake of food by fish (Margolis, 1953).

ACKNOWLEDGMENTS

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Standing Crop of Benthic Infauna in Puget Sound and Off the Coast of Washington[1]

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During the period May 28 to August 1, 1967, benthic infauna was collected at 37 stations in Puget Sound and off the coast of Washington. When large but rare species were excluded from the samples, the variability in standing crop (ash-free dry weight) among replicate samples was less than among means from different stations. The mean standing crop for the offshore stations was 1.92 g/m², which is comparable with the standing crop on the shelf in the Gulf of Alaska, but less than half the mean standing crop for the Puget Sound stations; the difference may be explained in part by differences, previously reported, in primary productivity of the water masses. For the offshore stations there was a weak trend of increasing standing crop with depth. The standing crop at the shallow-water offshore stations, in substrates characterized as fine sand, was dominated by crustaceans and small lamellibranchs, whereas at the deeper stations, in sediments with high percentages of silt and clay, polychaetes and echinoderms were the most important contributors.

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INTRODUCTION

QUANTITATIVE BENTHOS INVESTIGATIONS in the state of Washington have been performed in the vicinity of the Friday Harbor Laboratories and in Puget Sound (Shelford and Towler, 1925; Shelford et al., 1935; Wennekens, MS, 1959; Lie, 1968), but the communities of the continental shelf are still virtually unknown, and Thorson (1958) emphasized the lack of benthos investigations in the northwest. In 1967 benthos was collected in Puget Sound, Juan de Fuca Strait, and off the northwest coast of Washington, and the present paper reports on the distribution of the standing crop of benthic infauna in the area.

MATERIALS AND METHODS

The material was collected during the period May 28 to August 1 with a 0.2-m² van Veen grab weighing 50 kg, at 37 stations located in Puget Sound, in Juan de Fuca Strait, and off the Olympic Peninsula (Fig. 1). At 31 stations three replicate samples were collected, at 5 stations two, and at 1 station only one sample. The bottom types varied from gravel to soft mud, and the stations ranged in depth from 12 to 329 m (Table 1). The stations off Seattle and in Holmes Harbor (stations A–G) will be referred to below as the Puget Sound stations, and the stations in Juan de Fuca Strait and off the Olympic Peninsula (stations 1–30) will be referred to as the offshore stations. The contents of the grab samples were sieved through stainless steel screens with 1 mm mesh size, and the fauna was preserved in 5–10% formaldehyde previously buffered with NaH_2BO_3.

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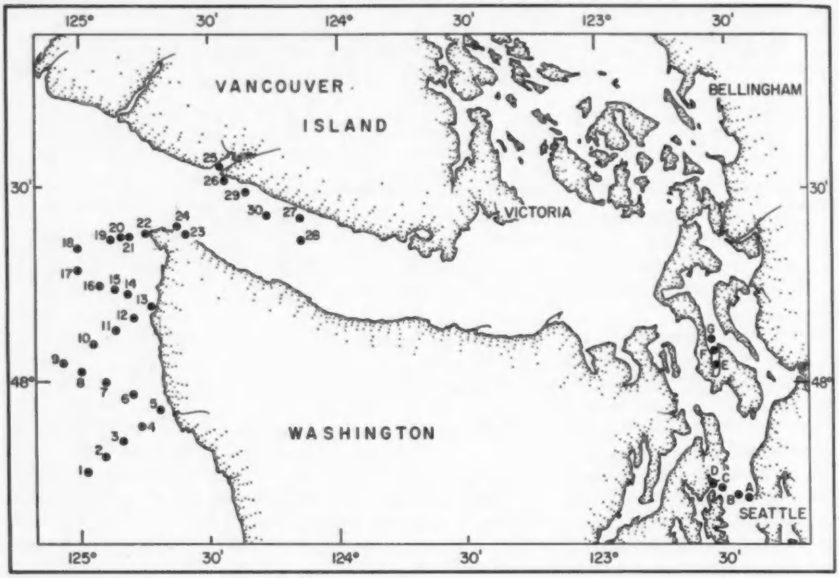

FIG. 1. Benthos stations in Puget Sound and off the coast of Washington.

In the laboratory the samples were sorted into Polychaeta, Crustacea, Mollusca, Echinodermata, and "Miscellaneous," and the wet weights for the various groups were determined after blotting on filter paper for 5–10 min. The wet weights were converted into ash-free dry weight following conversion factors determined by Lie (1968). The mean conversion factor for polychaetes was 0.13, for crustaceans 0.15, for lamellibranchs 0.05, and for echinoderms from 0.03 to 0.12. The conversion factor for converting total wet weight into ash-free dry weight is probably near 0.10. The wet weights of the group "Miscellaneous" were not converted into ash-free dry weight, because except at a few stations this group played an insignificant part in the total standing crop. At the stations where "Miscellaneous" was of any importance, the major part of the weight was contributed by species belonging to the epifauna (e.g., Anthozoa and Brachiopoda), which is not within the scope of this paper. The standing crop referred to below, therefore, represents the sum of ash-free dry weights of Polychaeta, Crustacea, Mollusca, and Echinodermata.

Considerable differences in standing crop among replicate samples of benthos occur (Holme, 1953), which may obscure real differences between localities. Sanders (1956) found that this variability was greatly reduced if large and rare organisms were excluded from the samples before weighing. These organisms may be so rare that their abundance based on the more-or-less accidental occurrence in a sample may be greatly overestimated. Furthermore, these organisms are often long-lived and slow-growing and their contribution to the turnover of organic matter may be out of proportion to their standing crop. In the present study the polychaete *Travisia pupa*, the lamellibranch *Compsomyax subdiaphana*, the echinoid *Brisaster latifrons*, and some large holothurians were omitted unless they occurred in all three replicates. Such adjustments of the weights were performed at 13 out of the 37 stations, and singly occurring *B. latifrons* was the most frequently removed species. Soviet benthos literature often distinguishes between total benthos biomass and the part of the biomass that can be utilized as food for fishes (Neiman, 1960). Large echinoids, asteroids, ophiuroids, and lamellibranchs measuring more than 45 mm are therefore excluded. This "usable" biomass, when converted into ash-free dry weight by conversion factors, becomes very similar to the standing crop of the present paper.

TABLE 1. Particle size analysis by per cent weight of the sediments. Offshore stations grouped as in Table 2.

Station	Depth (*m*)	Gravel (>2.0 mm)	Sand (0.063–2.0 mm)	Silt (0.031–0.063 mm)	Clay (<0.031 mm)	Mean particle size (*mm*)
			Puget Sound			
A	23	0.24	83.46	12.26	3.97	0.113
B	195	–	6.84	58.65	34.51	0.008
C	22	6.30	81.26	8.26	4.18	0.235
D	12	0.35	68.31	29.58	2.09	0.090
E	39	0.14	91.35	5.13	3.20	0.190
F	51	0.13	72.70	16.59	10.58	0.104
G	56	–	22.23	42.67	35.10	0.014
			Group I			
5	13	–	89.46	10.14	0.42	0.100
23	15	0.08	99.54	0.23	0.15	0.280
24	40	0.06	99.11	0.70	0.09	0.138
13	18	0.81	97.93	1.14	0.31	0.320
25	15	–	78.02	21.75	0.23	0.084
14	36	–	95.40	4.58	0.02	0.100
6	43	–	94.64	4.65	0.72	0.108
12	43	0.15	96.28	3.32	0.26	0.110
15	65	0.01	94.36	3.79	1.84	0.117
4	50	0.02	95.41	3.79	0.79	0.115
11	54	0.01	96.81	2.16	2.03	0.126
			Group II			
20	164	–	56.34	35.53	8.13	0.063
1	126	–	30.68	57.17	12.16	0.032
16	140	0.05	38.09	52.35	9.51	0.042
28	155	3.89	81.62	10.91	3.58	0.230
9	128	26.92	36.75	29.35	6.98	0.215
22	69	41.11	53.57	4.62	0.73	1.000
2	104	0.01	33.50	57.07	9.41	0.041
8	117	0.02	32.49	59.55	7.95	0.045
10	110	0.04	68.51	26.27	5.18	0.074
18	317	1.17	56.32	30.11	12.40	0.067
19	329	21.74	23.71	41.33	13.22	0.094
17	146	45.23	30.67	16.99	7.13	0.420
			Group III			
27	50	0.45	60.20	34.52	4.84	0.078
29	60	0.03	78.64	18.56	2.77	0.104
30	123	–	60.39	35.03	4.58	0.062
21	122	0.09	78.92	16.99	4.00	0.074
26	155	15.72	58.95	21.43	3.89	0.180
3	81	0.09	79.20	17.56	3.15	0.135
7	83	–	93.44	5.06	1.50	0.130

RESULTS AND DISCUSSION

Considerable differences in standing crop among replicate samples occurred even after large and rare species had been removed, but an analysis of variance showed that the differences in mean standing crop among the stations were statistically significant ($F = 15.19$, $F_{0.01} = 1.96$). The standing crop was very high at the two Puget Sound stations G and B, being dominated by the lamellibranch *Compsomyax subdiaphana* and the echinoid *Brisaster latifrons*, respectively (Fig. 2). Of all possible pairs of stations, 79% were significantly different in standing crop, i.e., the 95% confidence levels for single observations did not overlap the means.

The mean standing crop for the Puget Sound stations when stations G and B were excluded was still considerably higher than the mean for the offshore stations, 3.54 g/m^2 vs. 1.92 g/m^2. The standing crop of benthos is determined

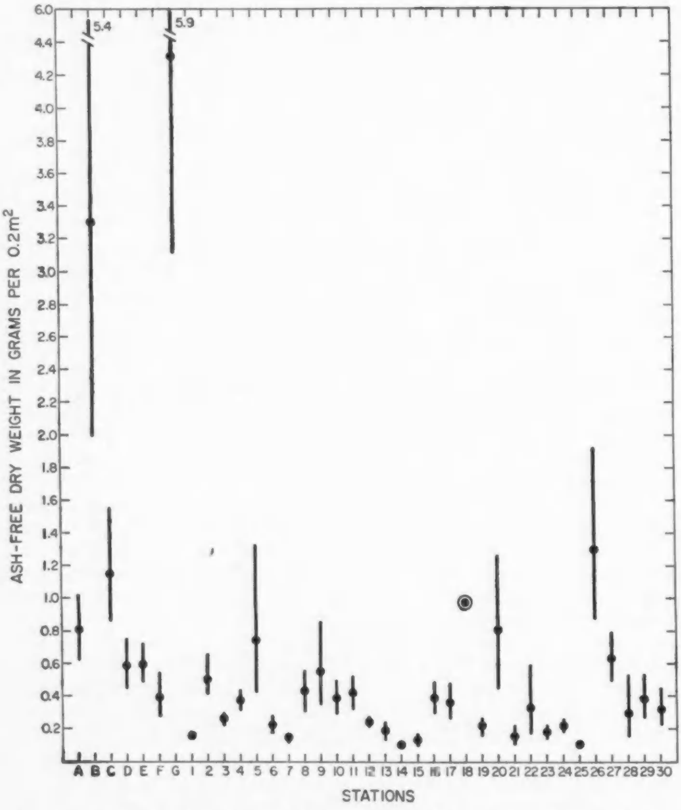

FIG. 2. Mean ash-free dry weights of benthic infauna in grams per 0.2 m^2 at 37 stations, with confidence interval estimates for single observations, $P = 0.95$. At station 18 (circled) the weight was based on only one sample.

by a number of factors such as age structure, growth rate, and predation, but there is to some extent a relationship between primary production of the overlying water masses and the standing crop of benthos. Raymont (1947, 1950) showed that fertilization of water masses resulted in a sharp increase in phytoplankton production and subsequently also an increase in standing crop of benthos. The annual primary productivity of the phytoplankton off the coast of Washington (Anderson, 1964) ranged from 61 g C/m^2 in the oceanic waters to 158 g C/m^2 in the upwelling areas, as compared with 268 and 459 g C/m^2 at two permanent stations in Puget Sound in 1964 (George C. Anderson, personal communication). This difference in primary production may in part account for the difference in standing crop of benthos from the two areas.

The standing crop at the stations A–D was considerably lower in the 1967 study than in August 1964, when the standing crop at the same four stations was measured (Lie, 1968). At station A the reduction was from 7.56 to 4.04 g/m^2, at station B from 18.86 to 16.50 g/m^2, at station C from 9.81 to 5.72 g/m^2, and at station D from 9.65 to 2.91 g/m^2. Long-term studies are required to evaluate the causes and eventual periodicity of these fluctuations.

Comparisons of standing crop from different studies must be viewed with caution because of the varying techniques of sampling and treatment of samples, but the standing crop of benthic infauna off the coast of Washington appears to be lower than those reported in comparable studies in European waters and on the east coast of the United States (Sanders, 1956). It is also considerably lower than the standing crop observed off Santa Barbara in California (Barnard and Hartman, 1959), which even after excluding the large holothurian *Molpadia intermedia* and the echiuroid *Listriolobus pelodes* was still from 10 to 20 g/m^2. In the Gulf of Alaska Shevtsov (1964) found a mean "usable" biomass of 80 g/m^2, which would correspond to a standing crop of about 8 g/m^2. However, the mean was heavily influenced by extremely high values at 4 of his 30 stations. When these four stations were excluded, the mean standing crop of the "usable" biomass was 2.7 g/m^2, and at 50% of the stations the standing crop was equal to or less than 2 g/m^2. The size of the standing crop off the coast of Washington, therefore, seems to be comparable with the standing crop in the Gulf of Alaska.

There was no correlation between depth and standing crop when all stations were considered, but when the Puget Sound stations were excluded there was a weak tendency of increasing standing crop with increasing depth. The rank correlation coefficient, τ (Kendall, 1962), between depth and standing crop for the offshore stations was 0.28, which was significant at $P = 0.05$. The rank correlation coefficient between standing crop and mud (silt + clay) percentage of the sediments was also 0.28. However, mud percentage and depth for the offshore stations were rather strongly autocorrelated ($\tau = 0.47$, $P < 0.01$) and therefore one cannot determine whether the increasing standing crop is related to increasing depth or mud content of the sediments.

There were distinct differences among the offshore stations in the relative contribution to the standing crop by the various taxonomic groups (Table 2). One group of stations was characterized by high percentages of both crustaceans

TABLE 2. Per cent contributions to the standing crop by the four major taxonomic groups. Stations arranged according to decreasing sums of Crustacea and Mollusca in group I, decreasing percentages of Polychaeta in group II, and decreasing sums of Polychaeta and Mollusca in group III.

Station	Polychaeta	Crustacea	Mollusca	Echinodermata	Standing crop (g/m^2)
			Group I		
5	4.56	71.93	23.39	0.10	4.275
23	7.56	27.73	64.71	0	1.190
24	7.28	47.09	45.15	0.50	1.030
13	18.18	41.21	40.60	0	0.825
25	17.92	24.53	56.60	0.94	0.530
14	20.21	27.26	48.94	3.19	0.470
6	24.35	22.17	46.09	7.39	1.150
12	19.18	10.61	55.10	15.10	1.225
15	23.14	30.58	33.88	12.40	0.605
4	41.89	17.57	32.43	8.10	1.480
11	23.74	16.55	27.58	32.13	2.085
Mean	18.91	30.69	43.13	7.25	1.351
			Group II		
20	94.48	1.32	4.08	0.10	4.170
1	86.67	2.67	10.67	0	0.750
16	83.98	2.58	9.30	4.13	1.935
28	82.93	5.49	3.96	7.62	1.635
9	66.49	2.08	12.67	18.75	2.880
22	65.04	13.28	16.53	5.15	1.845
2	63.31	7.87	12.50	16.33	2.480
8	54.48	2.83	15.57	27.12	2.120
10	53.09	1.03	11.34	34.53	1.940
18	46.37	1.02	12.97	39.63	4.895
19	35.38	1.89	3.77	58.96	1.060
17	33.65	21.79	7.69	36.86	1.560
Mean	63.82	5.32	10.08	20.76	2.272
			Group III		
27	29.78	2.78	65.74	1.70	3.240
29	43.68	1.52	51.77	3.03	1.980
30	19.82	2.37	68.34	9.47	1.690
21	45.56	2.96	38.46	13.02	0.845
26	33.71	9.31	48.80	8.18	6.660
3	37.35	7.39	38.13	17.12	1.285
7	24.63	8.46	48.46	18.46	0.650
Mean	33.50	4.97	51.38	10.14	2.335

and molluscs. These stations are distributed along the coast offshore and on exposed beaches in Juan de Fuca Strait (Fig. 1) in depths from 13 to 65 m and with substrates dominated by fine sand (Table 1). The second group of stations had very high percentages of polychaetes and sometimes echinoderms. These stations were found on the outer part of the continental shelf (Fig. 1) in depths from 104 to 329 m and with substrates dominated by high percentages of silt and clay. Two of the stations in the group (22 and 28) that were located in Juan de Fuca Strait and close to the beach near Cape Flattery differed from the rest of the stations in their low percentages of silt and clay (Table 1). The standing crop of the third group of stations was dominated by high percentages of molluscs and low percentages of crustaceans. Four of these stations were located in Juan de Fuca Strait and three off the coast in depths from 50 to 155 m and with substrates not significantly different from those of the second group.

The mean standing crop for the three groups of stations was 1.351, 2.272, and 2.335 g/m^2, respectively, but the differences between the means were not significant at $P = 0.05$ when tested by a t-test.

The standing crop at the first group of stations was dominated by small crustaceans with a life span of about 1 year (Thorson, 1957) such as the cumacean *Diastylopsis dawsoni* and the amphipods *Ampelisca macrocephala*, *Paraphoxus abronius*, and *Paraphoxus obtusidens*, whereas at the other two groups of stations the standing crop was dominated by species with a life span of 2 years or more, such as large polychaetes of the families Maldanidae and Ophelidae; the ophiurids *Amphiodia urtica*, *Amphioplus* sp., and *Ophiura lutkeni*; and the lamellibranchs *Yoldia ensifera* and *Macoma* spp. The production of organic matter by the macrobenthos at the first group of stations may therefore be as high as or higher than at the other two groups in spite of the lower standing crop.

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Hypoxial Stress Prior to Emergence and Competition Among Coho Salmon Fry

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MASON, J. C. 1969. *Hypoxial stress prior to emergence and competition among coho salmon fry.* J. Fish. Res. Bd. Canada 26: 63–91.

Competition among coho salmon fry in stream aquaria supplying natural drift was found to reflect the history of exposure of eggs and resulting fry to dissolved oxygen concentration prior to emergence. Size disparities induced by differential hypoxial stress were amplified with time, and fry that had been exposed to the most severe hypoxial conditions were most prone to emigrate. Most emigrants placed in an initially vacant, replicate system remained there, grew rapidly, and became as large as, or larger than, nonemigrants. Size of former emigrants reflected enhanced feeding opportunity due to less competition for food and space in the replicate system. Competition was referred more precisely to hypoxial history by using net production. A replicate population fed hatchery food provided a comparison. When exposed to the stream aquaria, this population substantiated the previous findings. Diel cycles of activity and aggression peaked at dawn and dusk, and were related to competition for food and space. The ecological significance of the results is discussed with particular regard to competition for food and space, and the effect of a size-related social order that put smaller individuals at a disadvantage.

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INTRODUCTION

PHYSICOCHEMICAL CONDITIONS in the gravels used by spawning salmonids are frequently inimical to high survival. Less than 10% of the eggs deposited by chum (*Oncorhynchus keta*), pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) in streams of British Columbia and Alaska usually leave the redds as free-swimming fry (Royce, 1959). In a recent study, Koski (MS, 1965) determined that mean survival to emergence was 27% for coho fry (*O. kisutch*) from redds in three small streams.

The oxygen supply to embryos and fry in the redds may largely determine survival and depends on interrelationships between gravel permeability, water velocity, and dissolved oxygen concentration (Wickett, 1954, 1958, 1962; Phillips and Campbell, 1961; Coble, 1961; McNeil, MS, 1962).

Decreased oxygen availability induces hypoxial stress[2] ultimately leading to developmental abnormalities (Garside, 1959) and embryo mortality when

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[2] In this paper, the term "stress" is used in the generalized sense proposed by Brett (1958) who defined stress as "a state produced by any environmental or other factor which extends the adaptive response of an animal beyond the normal range, or which disrupts the normal functioning to such an extent, that, in either case, the chances of survival are significantly reduced."

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the compensatory ability of the embryo to reduce its metabolism is exceeded. Hatching delay, premature hatching, and lengthening of the hatching period can also be induced by restricted oxygen supply. Size of surviving embryos at hatching is decreased by restricted oxygen supply (Silver et al., 1963; Shumway et al., 1964) and may be accompanied by general debility, depending on the degree of hypoxial stress imposed.

Among members of a brood year, those surviving hypoxial stress which also succeed in leaving the gravel may be at a disadvantage when competing with other fry that have experienced more favorable conditions in the redds. For example, Mason and Chapman (1965) found that first-emerging coho fry became ecological dominants in populations of coho fry residing voluntarily in stream aquaria. They grew more rapidly and were more prone to remain in residence than fry which emerged later.

Therefore, hypoxial stress was imposed on coho embryos and resulting fry until yolk absorption and measured in terms of survival and growth. Further disadvantage among these fry was then investigated in stream aquaria after yolk absorption in terms of growth, net production, emigration, distribution patterns, and aggressive behavior.

MATERIALS AND METHODS

INCUBATION APPARATUS AND PROCEDURE

Eggs from one female were fertilized with milt from two males and incubated in standard hatchery baskets for 15 days at an average water temperature of 7.5 C.

They were then divided into four groups of about 400 eggs each and placed in two layers on the middle platforms of the incubation chambers in the incubation apparatus (Fig. 1). This apparatus consisted of temperature regulating reservoirs, deoxygenation columns, flow regulators, and incubation chambers. A full description of this apparatus is given by Mason and Fessler (1966).

During the 24-hr period immediately after, the apparatus was gradually adjusted to supply water at an apparent velocity of 223 cm/hr to the incubation chambers and at concentrations of 11 (control), 5, and 3 (duplicated) mg/liter of dissolved oxygen. The lowest oxygen concentration was duplicated to insure survival of sufficient alevins for further experimentation. Operation levels of water flow, temperature, and dissolved oxygen concentration are given in Table 1.

In the 5 and 3 mg/liter chambers, water temperatures were adjusted upward to compensate for the anticipated delay in development resulting from lowered oxygen concentration, so that embryos and resulting fry subjected to the three treatments would complete vitellus absorption at the same time. The amount of temperature increase was calculated from unpublished data of Phillips[3] and amounted to 0.72 degree C for the 5 mg/liter and 1.84 degrees C for the 3 mg/liter treatments. Further minor temperature adjustments were made during the last 2 weeks prior to yolk absorption to rectify small differences in degree of yolk absorption among the three groups. These differences were determined visually.

Two days after complete hatching, samples of 25 fry were removed from each incubation chamber (samples from the two chambers receiving 3 mg O_2/liter were pooled and 25 fry were withdrawn) and the following measurements were made: fork length (determined under a dissecting microscope on anaesthetized fry), mean total volume (fry plus yolk) and mean fry volume (minus yolk) by displacement, mean dry weights of fry and separated yolk after drying to constant weight at 80 C.

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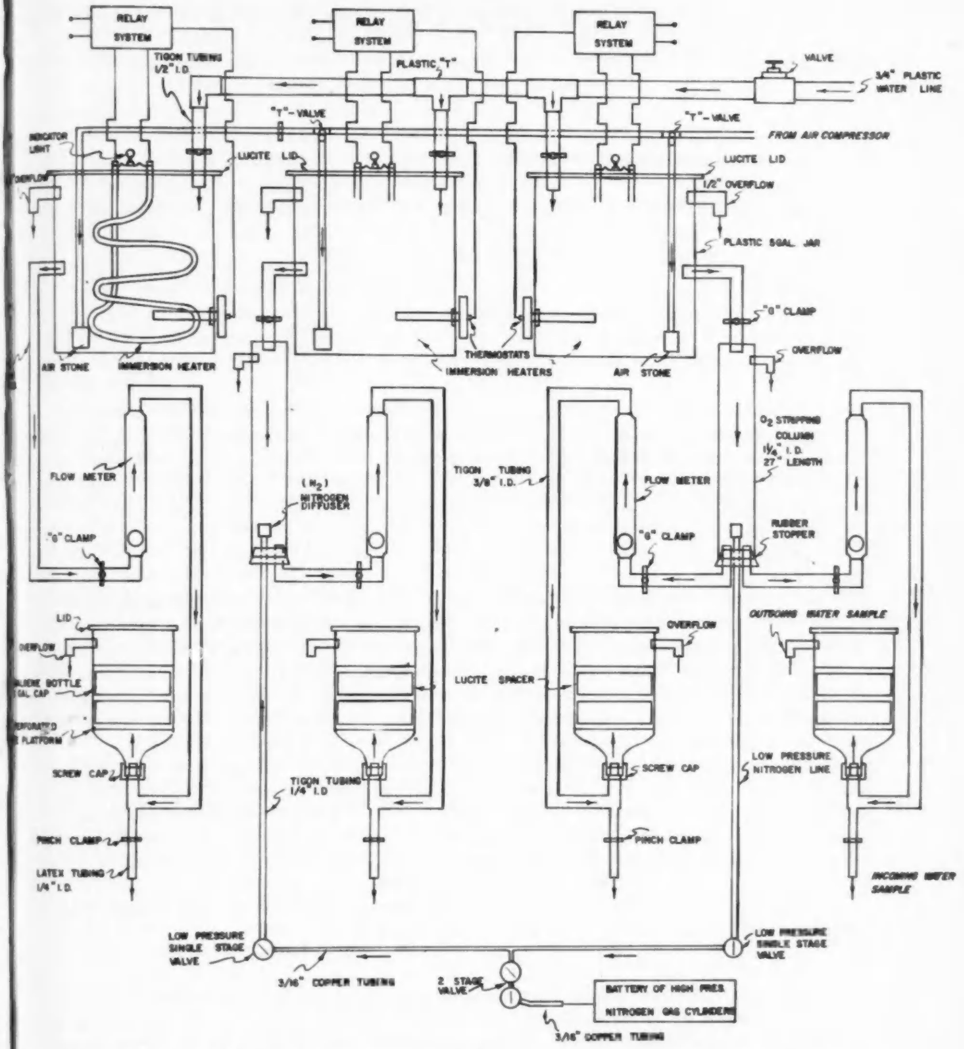
[3] Personal communication from Robert W. Phillips, Division of Wildlife Research, Oregon State Game Commission, Ore.


RELAY SYSTEM
RELAY SYSTEM
RELAY SYSTEM
TIGON TUBING 1/2" I.D.
PLASTIC "T"
VALVE
3/4" PLASTIC WATER LINE
"T"-VALVE
"T"-VALVE
FROM AIR COMPRESSOR
INDICATOR LIGHT
LUCITE LID
LUCITE LID
LUCITE LID
OVERFLOW
1/2" OVERFLOW
PLASTIC 5GAL. JAR
"G" CLAMP
AIR STONE
IMMERSION HEATER
THERMOSTATS
IMMERSION HEATERS
AIR STONE
OVERFLOW
O2 STRIPPING COLUMN 1¼" I.D. 27" LENGTH
FLOW METER
TIGON TUBING 3/8" I.D.
(N2) NITROGEN DIFFUSER
FLOW METER
"G" CLAMP
"G" CLAMP
RUBBER STOPPER
LID
OVERFLOW
UERE BOTTLE GAL CAP
PERFORATED E PLATFORM
LUCITE SPACER
OVERFLOW
OUTGOING WATER SAMPLE
SCREW CAP
SCREW CAP
TIGON TUBING 1/4" I.D
LOW PRESSURE NITROGEN LINE
PINCH CLAMP
PINCH CLAMP
LATEX TUBING 1/4" I.D.
INCOMING WATER SAMPLE
LOW PRESSURE SINGLE STAGE VALVE
LOW PRESSURE SINGLE STAGE VALVE
3/16" COPPER TUBING
2 STAGE VALVE
BATTERY OF HIGH PRES. NITROGEN GAS CYLINDERS
3/16" COPPER TUBING

1. Schematic diagram of the incubation apparatus employed to subject coho embryos and resulting fry to three concentrations of dissolved oxygen prior to yolk absorption.

STREAM AQUARIA AND HOLDING TANK

Test fry were placed in two aquaria simulating stream conditions (Mason and Chapman, 1965) or in a circular aluminum tank. Fish in the tank received a hatchery diet; those in the aquaria relied entirely on stream drift in the water supply.

Modifications to the stream aquaria were as follows. The gravity flow system was replaced by a low-head, high-capacity electrically powered pump in conjunction with a 3.8-m^3, constant-head reservoir. Intake pipes to the aquaria issued from the base of the reservoir on opposite

TABLE 1. Mean rates of water flow, concentrations of dissolved oxygen, and temperatures imposed on coho eggs and resulting fry in the incubation apparatus. Based on daily measurements.

Environmental factor	Dissolved oxygen (*mg/liter*)		
	11	5	3
Flow rate (*ml/min*)	518.4[a]	517.0	518.8
Range	430–530	449–569	449–550
SE[b]	6.5	5.5	4.5
Dissolved oxygen (*mg/liter*)	11.45	5.14	3.12
Range	11.2–12.5	4.3–6.0	2.6–4.0
SE	0.11	0.02	0.04
Temp (*C*)	8.84	9.56	10.68
Range	7.3–9.4	8.8–11.1	10.0–11.7
SE	0.06	0.06	0.01

[a] Equivalent to an apparent velocity of 223 cm/hr based on cubic centimeter flow/hr per cm^2 of cross-sectional area including both solids and voids. Actual velocity at the plate pores was about 1180 cm/hr based on a porosity of 0.19 (19% of the plate area was composed of pores). The mean actual velocity past the eggs lay between these two values, probably at about one-half of the pore velocity.

[b] Standard error.

sides and flow into the aquaria was controlled by valves. Both aquaria were divided into four units consisting of alternating riffles and pools (Fig. 2). Water flow into the aquaria was held at 12.4 liters/sec and water depth in the riffles was set at 13 cm resulting in velocities of 24–27 cm/sec. To discourage avian predators and provide overhead cover, both stream aquaria were equipped with wooden frames covered with fine monofilament gillnetting. The netting was overlaid with pieces of black plastic sheeting cut into irregular forms and fastened to the netting so as to provide about 25% cover.

The aluminum tank had a capacity of 860 liters. Stream flow into the tank was controlled at 38 liters/min, allowing an exhange rate of 4.4%/min. The exchange rate in the aquaria was approximately 14 times greater than that in the circular tank. Fry were fed ground beef liver and meal at hourly intervals during the day for the first week, five times per day during the subsequent 2 weeks, and three times per day during the last 3 weeks of the experiment. Those fry in the aquaria relied entirely on stream drift in the incoming water supply.

Maximum and minimum mean weekly water temperatures, based on daily records, ranged from 9.1 to 16.8 C and from 7.1 to 13.7 C, respectively.

TREATMENT OF FRY AT EMERGENCE

Samples of 250 fry from each incubation treatment were retained for further experimentation. The fry reared at 11 mg O_2/liter (control) were marked by removing the right pelvic fin, using corneal surgical scissors and a dissecting microscope. The left pelvic fin was removed from

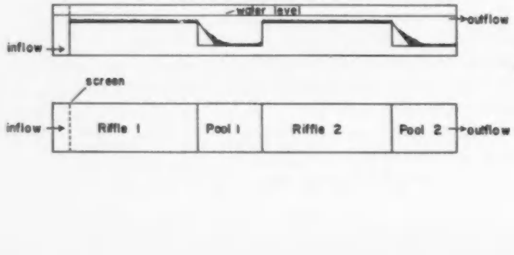


FIG. 2. The side and top aspects of each stream aquarium.

the 5 mg/liter fry and the 3 mg/liter fry were not marked. All fry were anaesthetized (M.S. 222, Sandoz), the 3 mg/liter fry being subjected to the same handling procedure as the other two except for excision of the fin. The treatment effect on the 3 mg/liter fry may have been attenuated somewhat since any detrimental effects of the fin excision on survival, growth, etc., were avoided.

Two days later, two lots of 110 fry each were separated from each of the three treatments of 250 fry. A total of 330 fry (one lot from each of the three treatments) was placed in the left stream aquarium and henceforth is referred to as population I. The remaining 330 fry formed a control population (unlimited food supply) designated population II, and were put in the circular tank.

TREATMENT OF FRY IN THE STREAM AQUARIA

Daily records were kept of fork length (to the nearest 0.1 mm), live weight (to the nearest 0.1 mg), and mark of individual fry emigrating[4] from the stream aquaria. After a 1-hr holding period to allow recovery from anæsthesia, emigrants from the left stream aquarium were transferred to the right one. Emigrants from the right system were similarly processed but released into the river after recovery.

Spatial distribution in the two aquaria was recorded weekly from dawn to dusk at hourly intervals or more frequently by counting fish in the pools and riffles and noting their marks.

Aggressive behavior was recorded weekly in both aquaria. Individual fish in each riffle and pool were observed during 1-min intervals until 45 min of observation time had elapsed, making a total of 6 hr of observation per week. When the number of fish in particular riffles and pools did not exceed 45, care was taken to distribute observation time in an equitable manner. Aggression was recorded in five categories forming a gradient in intensity of aggression culminating in "nip contact". Their description follows: (*a*) *Nip contact* — movement toward adversary followed by a nip that appeared to make contact. (*b*) *Nip close* — movement toward adversary followed by a nip that did not appear to make contact but occurred within about one head length of the adversary. (*c*) *Nip toward* — movement toward adversary followed by a nip that was made more than one head length away from the adversary. (*d*) *Drive toward* — movement toward adversary but mouth remained closed. Interaction was usually terminated by the adversary moving away promptly. (*e*) *Threat display* — behavior characterized by erection of paired and median fins, lowering of the basihyal apparatus, expansion of the opercles, and an open mouth. Display was recorded in three subcategories: (e_1) *Horizontal display* — body axis horizontal. (e_2) *Head down display* — body axis varying from the horizontal to about 80 degrees. Occurred in both high and low water velocities and frequently accompanied by slow caudal undulations termed "wig-wag display" by Hartman (1965). (e_3) *Head up display* — body axis reversed to (e_2) varying in angle from the horizontal to nearly 90 degrees. Occurred only in pools at low water velocities and sometimes accompanied by slow spasmodic caudal undulations unlike those in (e_2).

After 6 weeks, population I was removed from the stream aquaria and fork length, live weight, and mark were recorded for all fry. Population II (in the circular tank) was treated likewise, given 1 day to recover, then put in the left stream aquarium. Emigrants were treated as described before.

The performance of population II in the stream aquaria provided partial replication of the experiment.

RESULTS AND DISCUSSION

PERFORMANCE IN THE INCUBATION APPARATUS

Developmental delay associated with hypoxia was overcome by 66 additional temperature units (1 C.T.U. = 1 degree C/day) for the 5 mg/liter fry, and 168 units for the 3 mg/liter fry. The saturation fry required 775 C.T.U. to complete development.

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[4] As used here, the term "emigrating" signifies downstream passage from the stream aquaria into a Wolfe-type trap. No physioecologic connection with the synonym "migration" is intended.

Survival of both embryos and fry was related directly to degree of hypoxial stress applied, highest mortalities being associated with the lowest dissolved oxygen concentration (Table 2). Most mortality occurred prior to hatching but, at 3 mg O_2/liter, fry mortality averaged 33% of total mortality. Of nearly 1500 fry hatching from surplus eggs held in a hatchery basket, 1% died after hatching. Therefore, some mortality of fry in the apparatus may have been due to handling. Egg mortality in the hatchery basket was not recorded.

TABLE 2. Mortalities of coho embryos and fry up to time of yolk absorption according to concentration of dissolved oxygen in the incubation apparatus.

Mean dissolved oxygen (*mg/liter*)	No. of eggs	% mortality		
		Embryo	Fry	Total
11.45	429	15.2	2.1	17.2
5.14	405	21.0	1.7	22.7
3.12				
a	399	26.8	15.3	42.1
b	396	28.0	11.6	39.7

Discounting hatching rates of less than five fry per day, the duration of the hatching period was least at 11 mg/liter, being 7, 8, and 10 days, respectively, for the three oxygen concentrations (Fig. 3). The highest hatching rate also took place at 11 mg/liter, reaching 135 fry/day.

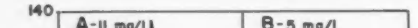

FIG. 3. Temporal patterns of hatching of coho eggs subjected to three concentrations (A–C) of dissolved oxygen in the incubation apparatus. D — incubated in a hatchery basket at river temperature and dissolved oxygen concentration (air saturation), hatching began 18 days later in D than in experimental groups due to lower water temperatures during incubation.

Size of fry after hatching was greatly reduced at the lowered oxygen concentrations in terms of length, volume, and dry weight (Table 3). Differences in mean length were highly significant ($P < 0.01$). Volume and dry weight values indicate considerable loss in yolk conversion efficiency at 3 mg/liter. The 5 mg/liter fry weighed twice as much as the 3 mg/liter fry yet retained nearly as much yolk as did the latter. Both yolk weight and total weight of the 5 mg/liter fry were greater than those of the 11 mg/liter fry though the 5 mg/liter fry weighed less. Mean volume of eggs just prior to hatching was 0.287 cc. Therefore, about 5% of egg volume was lost at hatching of eggs exposed to 5 and 11 mg/liter and 16.7% for eggs exposed to 3 mg/liter.

TABLE 3. Size statistics at hatching for coho fry from eggs subjected to three concentrations of dissolved oxygen in the incubation apparatus. Based on samples of 25 fry each taken 2 days after complete hatching. Values in parentheses are percentages.

Measure	Dissolved oxygen (*mg/liter*)		
	11	5	3
Length (*mm*)			
Mean	28.12	25.42	22.88
Range	24.7–29.7	24.6–27.1	19.7–24.1
SE[a]	0.27	0.20	0.21
Mean vol (*cc*)			
Fry	0.117(43)	0.079(29)	0.49(20)
Yolk	0.155(57)	0.192(71)	0.190(80)
Total	0.272	0.271	0.239
Mean dry wt (*mg*)			
Fry	14.7(17)	10.1(11)	5.3(6)
Yolk	69.9(83)	83.1(89)	84.8(94)
Total	84.6	93.2	90.1

[a] Standard error.

Upon absorption of the vitellus, differences in mean length remained highly significant ($P < 0.01$) although the difference between the 11 mg/liter and 5 mg/liter fry was markedly reduced (Table 4). The 3 mg/liter fry were much smaller, there being nearly 5 mm difference in body length between them and the 11 mg/liter fry. The "K" values (based on fork length) revealed that the 3 mg/liter fry were in the best condition and the 11 mg/liter fry in the worst, although the difference between the 11 mg/liter fry and 5 mg/liter fry was not great. Yolk conversion efficiency was high in all three groups but highest for the 11 mg/liter fry.

PERFORMANCE OF POPULATION I IN THE STREAM AQUARIA

After a 5-day familiarization period, the outlet screen was removed from the left stream aquarium and daily emigration recorded. Trends in emigration were soon established. Rate of emigration reflected the degree of hypoxial

TABLE 4. Size statistics at time of yolk absorption for coho fry subjected to three concentrations of dissolved oxygen in the incubation apparatus. Based on samples of 30 fry each.

Measure	Dissolved oxygen (*mg/liter*) 11	5	3
Mean length (*mm*)	37.77	37.02	33.11
Range	36.0–39.7	35.8–38.6	30.8–34.7
SE[a]	0.18	0.14	0.21
Mean wet wt (*mg*)	436.0	417.4	326.9
Mean dry wt (*mg*)	81.0	79.4	76.0
% moisture	81.4	81.0	76.8
Dry wt loss[b] (*mg*)	3.6	13.8	14.1
Yolk conversion efficiency[b,c]	0.948	0.834	0.834
Coefficient of condition (K)	0.809	0.823	0.901

[a]Standard error.
[b]From hatching to time of yolk absorption.
[c]Based on dry weight.

stress imposed (Fig. 4). The 3 mg/liter fry left the aquarium at a much faster rate than did the other two groups of fry, the difference between the 5 and 3 mg/liter fry being greater than between the 11 and 5 mg/liter fry. Emigration trends remained largely stable until May 19 when a sudden acceleration in the emigration of 11 mg/liter coho took place. Of these fish 16 left in 3 days, and thereafter emigration consisted primarily of 11 mg/liter coho. The 5 and 3

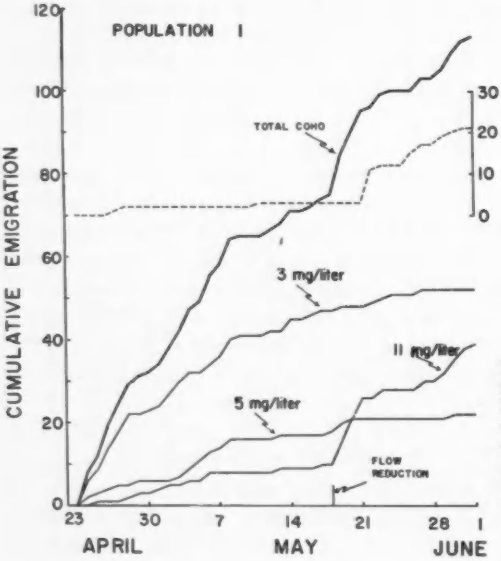

FIG. 4. Cumulative numbers of coho fry in population I emigrating from left (*solid line*) and right (*broken line*) stream aquaria according to oxygen concentrations in the incubation apparatus prior to yolk absorption; 110 fry in each group put in left aquarium, emigrants (113 in all) being placed in right aquarium.

mg/liter fish were affected little; emigration of 5 mg/liter fish increased but slightly during the same 3-day period. The sudden increase in emigration is attributed to reduction in flow resulting from an interruption in electrical service for 2 hr. During this time, the gravity flow system was used and provided about 20% of the normal flow.

A total of 113 coho entered the trap from the left aquarium and were transferred to the right aquarium. Few fish left this system (Fig. 4). Only 3 of 87 coho, all 3 mg/liter fish, had left the right stream aquarium by May 21, when an increase in emigration took place that predominantly involved the other two groups. These latter were probably some of the same individuals which were disturbed by flow reduction in the left aquarium on May 19. A 3-day delay in its reflection in the right system suggests that flow reduction did not greatly disturb resident coho here, and that the handling procedure accorded emigrants from the left system may have inhibited, or at least delayed, continued emigration.

Losses of emigrant biomass from the left system followed the numerical losses and served to emphasize the effect of flow disturbance on the 11 mg/liter coho. These fish composed nearly 62% of the biomass lost from the system.

Fry of the three treatments maintained distinct size disparities at emigration (Fig. 5) that reflected their developmental histories. Their rates of increase in size at emigration were also dissimilar. Where necessary, the curves in Fig. 5 were extended to show that, at termination of the experiment, the apparent mean sizes of recent emigrants were considerably less than those of residents. These disparities were greatest for the 3 mg/liter coho and least for the 11 mg/liter fish. There was a difference in mean length of 3.08 mm ($P < 0.01$) between the 11 mg/liter coho leaving the left system during the last 6 days

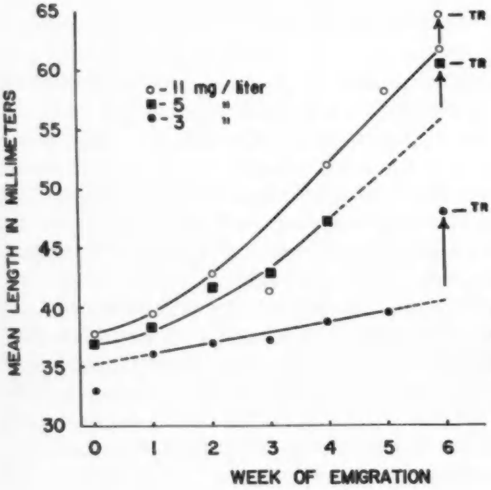

FIG. 5. Mean fork lengths of coho fry in population I that emigrated from the left aquarium. Broken lines are projections, curves fitted by eye. TR = terminal residents.

and 11 mg/liter fish in the terminal population of that system. Most probably, emigrants were smaller than residents throughout the experiment.

Instantaneous growth rates "g" (based on weight) of fish destined to emigrate were lower than those of fish which remained in residence throughout the experiment (Table 5, *upper*). The percentage difference in rate was 11 for the 11 mg/liter fish, 44 for the 5 mg/liter fish, and 66 for the 3 mg/liter fish.

Table 5. Instantaneous growth rates "g" (weight) on a daily basis for fry of the two populations in each aquarium according to concentration of dissolved oxygen prior to yolk absorption. The second set of values are standardized relative to "g" values for 11 mg/liter fry in the left aquarium.

Dissolved oxygen (*mg/liter*)	In left aquarium			Same emigrants in right aquarium
	Residents	Emigrants	Mean	
		Population I		
11	0.047	0.042	0.046	0.048
	1.02	0.91	1.00	1.04
5	0.043	0.024	0.041	0.064
	0.93	0.52	0.89	1.39
3	0.030	0.010	0.024	0.054
	0.65	0.22	0.52	1.18
		Population II		
11	0.020	0.010	0.018	0.021
	1.11	0.56	1.00	1.17
5	0.014	0.012	0.013	0.019
	0.78	0.67	0.72	1.06
3	0.010	0.008	0.009	0.013
	0.56	0.44	0.50	0.72

Chapman (1962) found that emigrants in natural populations of underyearling coho were consistently smaller than residents. For both resident and emigrant fish, reduced oxygen concentration during embryonic development was followed by lower instantaneous growth rates. After transfer to the right stream aquarium, the "g" values for the 3 and 5 mg/liter emigrants substantially exceeded that for the 11 mg/liter fish (both residents and emigrants).

Outlet traps were checked twice daily, usually at sunrise and sunset. Emigration was not related to the light cycle, although more coho entered the traps during daylight hours than at night. During the day 58% of the emigration took place, and daylight hours constituted about 60% of the 24-hr cycle.

The experiment was terminated on June 2 due to complete cessation of flow for 20 min while repairs were made in the water system. When the flow ceased, many coho began searching behavior and swam downstream into pool 2. Many fish swam upstream and began jumping vigorously at the screens when flow was resumed. Fourteen coho entered the trap for the left aquarium but none entered that for the right one. These trapped fish were put into pool 1 without delay. That evening, 11 coho were found in the left trap and 15 in the right one, prompting termination of the experiment on population I.

The 213 fish remaining in the left stream aquarium at the end of the experiment amounted to 65% of their initial number (Table 6). There were more resident 5 mg/liter coho (41% of the terminal population) than 11 mg/liter coho (33%) and 3 mg/liter coho (25%) were fewest. However, had the 11 mg/liter fish not responded as they did to the interruption of flow that ended the experiment, only about 14 of these fish would have left the system (based on extrapolation of the trend lines, Fig. 4) and the terminal value would have been 40% for the 11 mg/liter group in comparison with 32% for the 5 mg/liter group. Four fish, 3 mg/liter coho, were unaccounted for and were presumed to be victims of cannibalism.

Differences in length and weight among the three groups of fry at emergence were increased considerably in the aquarium (Table 6). The difference in length between 11 mg/liter and 5 mg/liter coho increased from 0.75 to 4.10 mm and the 11 mg/liter fish grew faster, in terms of both absolute and relative increases in length and weight, than the other two groups. Condition factors (K) generally increased but the initial ranking was reversed in the terminal population, the 11 mg/liter fish having the highest and the 3 mg/liter fish the lowest values.

In the right stream aquarium, the same ranking of size was maintained as in the left although the disparities in size were reduced (Table 6). On the average, the coho in the right aquarium were larger than those remaining in the left system due to accelerated instantaneous growth rates "g" that followed transfer to the right system (Table 5). Acceleration in growth rate was greatest for the 3 mg/liter fish (436%), less for the 5 mg/liter fish (167%), and least (11%) for the 11 mg/liter fish.

Percentage increases in length and weight were nearly equal for the 11 and 5 mg/liter coho, and slightly less for the 3 mg/liter group. Condition coefficients increased in all groups, terminal values being higher for 5 and 3 mg/liter fish than those for equivalent groups in the left system, and that for 5 mg/liter fish being higher than that for the 11 mg/liter group in either aquarium. Two fish, 3 mg/liter coho, were missing and presumed to be the victims of cannibalism.

The biomass relations of the three groups of coho were functions of both numbers of fish and their growth in the two stream aquaria. Loss of biomass from the left system remained fairly steady until the flow disturbance on May 21. Thereafter, it increased sharply and involved primarily the 11 mg/liter fish, the total biomass loss during the experiment being 99.6 g. Despite this loss, the terminal biomass for 11 mg/liter fish slightly exceeded that of the 5 mg/liter coho and composed nearly 45% (245.9 g) of the terminal biomass in the left system (Fig. 6, *upper*). The 3 mg/liter coho made up only 12% of the sum total of nearly 550 g.

In the right stream aquarium, most of the biomass loss seemed to involve fish that emigrated from the left aquarium when the flow was reduced on May 19

TABLE 6. Residence, emigration, growth, and production of coho fry of population I in the stream aquaria, according to concentration of dissolved oxygen prior to yolk absorption. Fish in right aquarium are emigrants transferred from left aquarium. Values in parentheses are percentages.

Dissolved oxygen (*mg/liter*)	Left aquarium			Right aquarium		
	11	5	3	11	5	3
Initial no.	110	110	110	See emigrant values for left aquarium		
Total fish		330				
Terminal no.	71(33.3)	88(41.3)	54(25.4)	21(26.9)	17(21.8)	40(51.3)
Total fish		213			78	
No. of emigrants	39(34.5)	22(19.5)	52(46.0)	18(54.5)	5(15.2)	10(30.3)
Total		113			33	
No. of missing fish	0	0	4	0	0	2
Initial mean length (*mm*)[a]	37.77	37.02	33.11	See values for left aquarium		
SE[b]	0.18	0.14	0.21			
Terminal mean length	64.73	60.63	48.02	64.80	63.41	55.46
SE	0.35	0.30	0.61	0.60	1.02	0.66
% increase	71.4	63.8	45.0	71.6	71.3	67.5
Initial mean wt (*g*)	0.4360	0.4174	0.3269	See values for left aquarium		
Terminal mean wt	3.4636	2.7081	1.2012	3.4504	3.2949	2.0588
SE	0.0686	0.0499	0.0565	0.1297	0.1779	0.8641
% increase	694.4	548.8	267.5	691.4	689.4	529.8
Coefficient of condition (K)						
Initial	0.809	0.823	0.901	See values for left aquarium		
Terminal	1.277	1.215	1.085	1.268	1.292	1.207
Input biomass (*g*)	48.0	45.9	36.0	61.6	18.0	19.9
Emigrant biomass (*g*)	61.6	18.0	19.9	33.3	5.7	4.8
Net production (*g*)	259.6	210.4	48.8	44.1	43.7	67.2

[a] Initial measurements were based on samples of 30 fry each. All fry were weighed and measured at termination.
[b] Standard error.

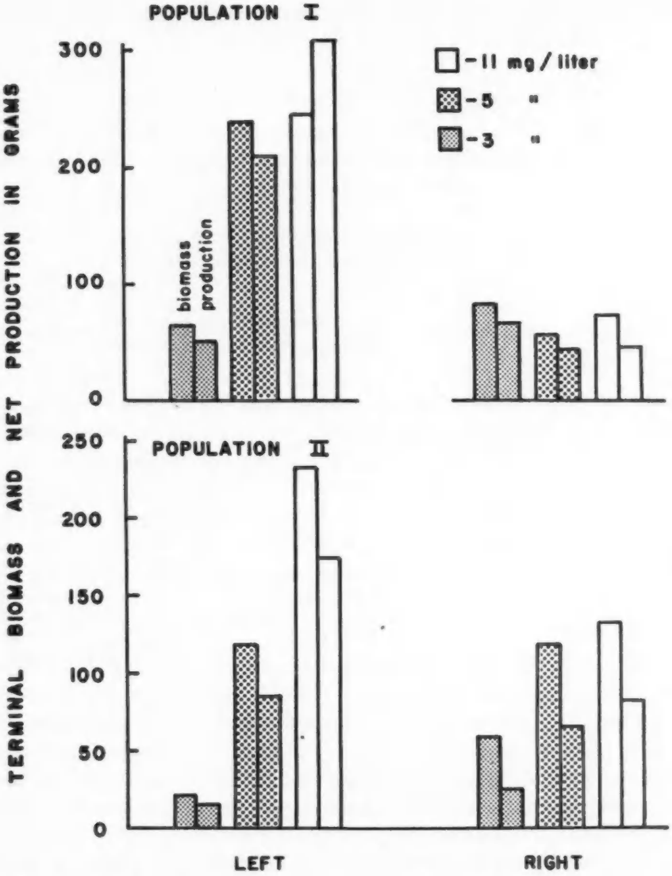

FIG. 6. Terminal biomass and net production of resident and emigrant coho fry in the left and right stream aquaria in relation to concentration of dissolved oxygen prior to yolk absorption. *Upper* — fry put in the left aquarium at yolk absorption (population I); *lower* — fry put in the left aquarium after an initial 6 weeks in a holding tank (population II) and upon removal of population I from the aquaria.

and stayed briefly in the right aquarium (Fig. 4), and 11 mg/liter fish made up 76% of the 43.8 g biomass lost. The 210.8 g of terminal biomass was composed of 34% 11 mg/liter coho, 27% 5 mg/liter coho, and 39% 3 mg/liter coho.

Net production in the systems was calculated for each group of fish by subtracting the initial or input biomass from the sum of the terminal and emigrant biomasses. Net production is the total increase in growth in the aquarium and is, therefore, the product of growth rate, number of fish, and time in residence. Net production (Table 6) amounted to 518.8 g in the left system and 155.0 g in the right one. Production was distributed as follows: 11 mg/liter coho, 50 and 29%, 5 mg/liter coho, 40 and 28%, and 3 mg/liter coho, 9 and 43% respectively, in the left and right system (Fig. 6, *upper*). Net production values are made slightly conservative by the loss of a few fish from the 3 mg/liter group (four fish in the left system and two in the right system — Table 6).

A production index using production by 11 mg/liter fish as a base was derived to facilitate comparison where

$$\text{production index} = \frac{\textit{per cent gain in biomass for given group}}{\textit{per cent gain in biomass for 11 mg/liter group}}$$

and where

$$\text{per cent gain in biomass} = \frac{\textit{net production in grams}}{\textit{initial or input biomass in grams}} \times 100$$

for each group of fry. The production index was 0.9 for the 5 mg/liter and 0.3 for the 3 mg/liter coho. The situation was reversed in the right system where the relative production of 3 mg/liter coho was nearly five times that of the 11 mg/liter value. This was due to the extensive emigration of 3 mg/liter fish from the left aquarium early in the experiment and greatly enhanced growth shown by these emigrants after transfer to the right system. Here, the production index was 3.4 for the 5 mg/liter fish and 4.7 for the 3 mg/liter fish.

GROWTH OF POPULATION II IN THE TANK

Fish reared in the tank (population II, Table 7) on hatchery rations did not grow as large as their counterparts in the stream aquaria (population I, Table 6) where 11 and 5 mg/liter coho gained about twice as much weight. Neither was there a significant difference among the three groups of coho in percentage increase in length, the range being 34.6–36.1%. This is in contrast to the situation in the left aquarium, where these values ranged from 71.4% (11 mg/liter) to 45.0% (3 mg/liter).

Growth in length while in the tank suggested that there was no competition for food. However, there was a gradient in relative weight increase though it was one weaker than that shown by population I in the left stream aquarium. Terminal mean lengths and weights remained significantly different ($P < .025$). The mean condition (K) of all three groups increased in the holding tank.

The nearly equal percentage increases in length for the groups of coho in the tank indicate that food competition began late in the holding period. Reduction in frequency of feeding from five to three times a week halfway through the holding period apparently operated to the advantage of the larger fish.

PERFORMANCE OF POPULATION II IN THE STREAM AQUARIA

After recovery from handling, 252 survivors were put in the left aquarium. The downstream screen was removed after 5 days to permit emigration.

Rate of emigration from the left aquarium was high at first but declined rapidly within 3 days to a low level that lasted for the rest of the experiment

TABLE 7. Initial[a] and terminal statistics on growth of coho fry on a hatchery diet in a circular tank (population II), according to concentration of dissolved oxygen prior to yolk absorption.

	Dissolved oxygen (*mg/liter*)		
	11	5	3
Initial no.	110	110	110
Total fish		330	
Terminal no.	107	103	86
Total fish[b]		296	
Initial mean length (*mm*)	37.77	37.02	33.11
SE[c]	0.18	0.14	0.21
Terminal mean length	51.39	49.84	44.89
SE	0.20	0.27	0.24
% increase	36.1	34.6	35.6
Initial mean wt (g)	0.4360	0.4174	0.3269
Terminal mean wt (g)	1.4007	1.2177	0.8262
SE	0.0846	0.0221	0.0015
% increase	221.3	191.7	152.7
Coefficient of condition (K)			
Initial	0.809	0.823	0.901
Terminal	1.032	0.984	0.913

[a] Based on samples of 30 fish. All fish were weighed and measured at termination.

[b] Some fish escaped due to an overflowing drain. Four dead fish (3 mg/liter) were removed from the tank.

[c] Standard error.

(Fig. 7). During the first 5 days, 85 coho entered the left trap and were transferred to the right system. Only 10 of these first occupants in the right system emigrated within 10 days and the subsequent emigration rate remained consistently low. Relative to their initial numbers, emigration rate was highest for the 3 mg/liter coho and lowest for the 11 mg/liter coho. Total emigrant numbers were equal (46 coho per group), being 49, 52, and 67% of initial numbers for the 11, 5, and 3 mg/liter coho, respectively.

At the end of the experiment, the 11 mg/liter fish formed the largest group in the left system although the fate of 13 coho remained unknown (Table 8). The disproportionately high number of missing 3 mg/liter fish suggests cannibalism. In the right stream aquarium, four 11 mg/liter and three 5 mg/liter coho are thought to have escaped from the trap.

In the stream aquaria, population II responded much in the same way as did population I. Size differences among the three groups of fry increased considerably despite the larger initial sizes when put in the stream systems. Emigrants increased their growth rates in the right system becoming larger than those remaining in the left aquarium. Their growth rates accelerated to a less marked degree than in population I (Table 5) but, in both populations, acceleration of growth was most extensive in 3 and 5 mg/liter fish. In further contrast to their counterparts in population I, the 11 mg/liter fish had the

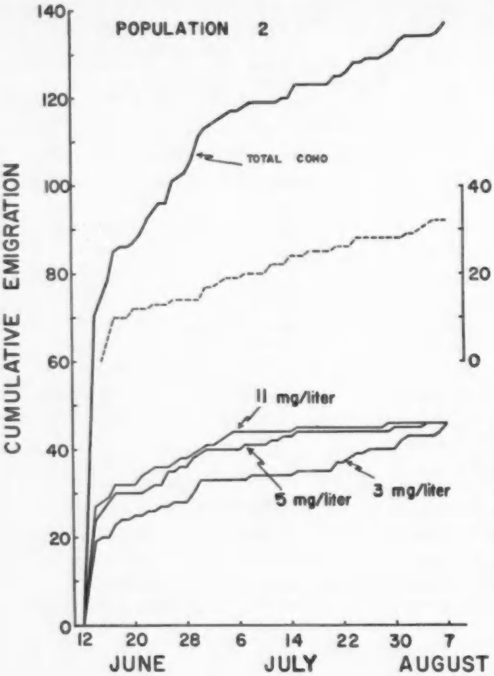

FIG. 7. Cumulative numbers of coho fry in population II emigrating from the left (*solid lines*) and right (*broken line*) aquaria, according to oxygen concentrations in the incubation apparatus prior to yolk absorption; see Table 8 for number of fry put in left aquarium after 6 weeks in the tank, emigrants (32 in all) being placed in right aquarium.

highest and the 3 mg/liter fish the lowest growth rate. The low "g" value for 11 mg/liter fish destined to emigrate was caused by several very small individuals. When the proportion of small fish was adjusted to match that for the 5 mg/liter group, a similar "g" value was obtained for the 11 mg/liter emigrants. Differences among the three groups of fry in length and weight were initially greater in population II than in population I and increased more markedly with time.

All condition cœfficients increased but were highest for the 11 mg/liter coho and lowest for the 3 mg/liter coho in both systems. They were lower than the corresponding values for population I (Table 6), due in part to the lower initial values for population II when put in the left stream aquarium.

Emigrant biomass trends paralleled the numerical trends, reflecting the size differences among the three groups of fish, and amounting to 203.9 g and 56.0 g for the left and right systems. Terminal biomass amounted to 370.1 g in the left system and 307.6 g in the right. Terminal biomass was distributed as follows: 11 mg/liter coho, 63 and 43%, 5 mg/liter coho, 32 and 38%, and 3 mg/liter coho, 6 and 19%, respectively, in the left and right systems (Fig. 6, *lower*).

Net production amounted to 271.3 g in the left system and 172.5 g in the right. Production was distributed as follows: 11 mg/liter coho, 64 and 47%,

TABLE 8. Residence, emigration, growth, and production of coho fry of population II in the stream aquaria, according to concentration of dissolved oxygen prior to yolk absorption. Fish in right aquarium are emigrants transferred from left aquarium. Values in parentheses are percentages.

Dissolved oxygen (*mg/liter*)	Left aquarium			Right aquarium		
	11	5	3	11	5	3
Initial no.	95(37.7)	88(34.9)	69(27.4)	45(34.9)	41(31.8)	43(33.3)
Total fish		252			129	
Terminal no.	47(46.5)	40(39.6)	14(13.9)	28(30.4)	30(32.6)	33(35.9)
Total fish		101			92	
No. of emigrants	46	46	46	14(43.8)	8(25.0)	10(31.3)
Total		138			32	
No. of missing fish	2	2	9	4	3	0
Initial mean length (*mm*)	51.73	50.15	44.84	See values for left aquarium		
SE[a]	0.27	0.28	0.29			
Terminal mean length	74.19	64.10	52.67	74.30	69.56	55.94
SE	1.51	1.22	1.07	1.73	1.64	0.69
% increase	43.4	27.8	17.5	43.6	38.7	24.8
Initial mean wt (*g*)	1.4417	1.2400	0.8216	See values for left aquarium		
SE	0.0943	0.0214	0.0167			
Terminal mean wt	4.9324	2.9209	1.5328	4.8717	3.9161	1.7765
SE	0.3790	0.2381	0.0936	0.3981	0.3266	0.0784
% increase	242.1	135.6	86.6	237.9	215.8	116.2
Coefficient of condition (K)						
Initial	1.042	0.983	0.911	See values for left aquarium		
Terminal	1.208	1.109	1.049	1.188	1.164	1.105
Input biomass (*g*)	137.0	109.1	56.7	76.7	68.4	46.1
Emigrant biomass (*g*)	78.2	75.7	50.1	26.6	15.4	14.0
Net production (*g*)	173.0	83.4	14.9	81.5	64.5	26.5

5 mg/liter coho, 31 and 37%, and 3 mg/liter coho, 6 and 15%, respectively, in the left and right system. Production index values were 0.6 for the 5 mg/liter coho and 0.2 for the 3 mg/liter coho in the left system, and 0.9 and 0.5 in the right. In the left system, population I had almost twice the production of population II whereas production in the right system was similar for both populations. In the left system, index values were relatively similar in both populations. In the right system, these values were much lower in population II and were similar to those for population I in the left aquarium, thus reflecting more intense competitive stress in population II.

Distribution in the Stream Aquaria

As reported previously (Mason and Chapman, 1965), underyearling coho concentrate toward the upstream ends of the aquaria, the majority of fish occupy pool 1, and the larger fish are usually found farthest upstream. In the present experiment, the average number of fish (derived from weekly counts) in the various areas of the aquaria indicate a similar pattern of distribution (Fig. 8).

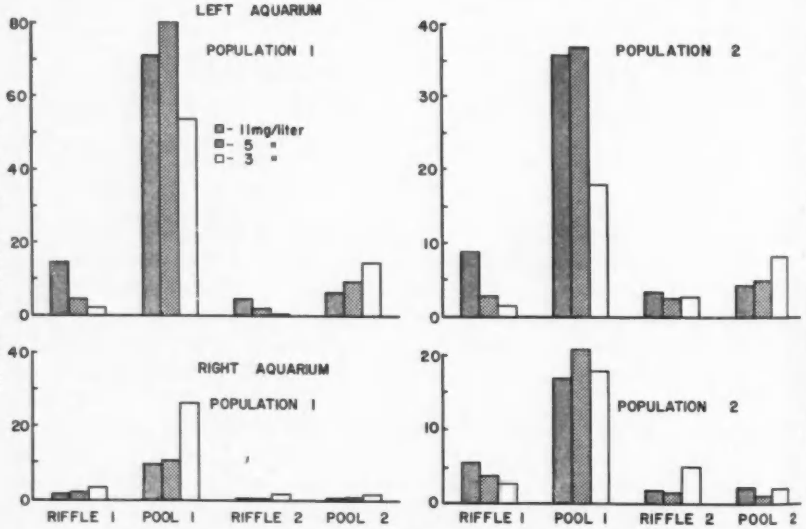

FIG. 8. Mean number of coho fry in both populations in each pool and riffle of the aquaria, according to concentrations of dissolved oxygen prior to yolk absorption.

The 11 mg/liter coho were more numerous than the other two groups in both riffles of the left aquarium (both populations) and in riffle 1 of the right one (population II). Consistent differences in distribution of the three groups of coho in three of the four riffles and pools can be seen by comparing weekly "observed" and "expected" percentage composition values for each group (Table 9). In general, the weekly distributions support the average differences

	Left aquarium					Right aquarium				
		Observed %					Observed %			
Week	Expected %[a] in each area	Riffle 1	Riffle 2	Pool 2	No. of fish observed	Expected % in each area	Riffle 1	Riffle 2	Pool 2	No. of fish observed
					Population I					
					3 mg/liter fish					
1	31	4	0	29	10	57	None – all fish in pool 1			0
2	28	1	2	37	67	60	None – all but one fish in pool 1			1
3	25	2	6	54	96	59	55	91	100	62
4	24	20	0	56	216	51	51	63	43	65
5	24	24	7	46	123	56	57	72	60	56
					5 mg/liter fish					
1	35	44	44	42	27	17		See above		0
2	36	30	50	36	129	16		See above		0
3	37	21	29	30	103	23	32	9	0	15
4	38	18	9	29	124	24	24	25	57	28
5	40	14	23	33	97	23	24	0	0	26
					11 mg/liter fish					
1	35	52	56	29	26	26		See above		0
2	36	68	48	27	166	24		See above		0
3	38	78	65	24	217	19	13	0	0	6
4	38	63	91	15	154	26	25	13	0	23
5	36	62	70	22	199	19	28	28	40	34
					Population II					
					3 mg/liter fish					
1	28	15	18	54	119	29	33	100	55	49
2	26	12	40	51	121	33	34	79	41	193
3	23	4	25	47	119	33	10	66	44	142
4	23	6	39	45	193	34	22	65	49	161
5	22	16	26	50	203	35	17	45	27	94
6	18	15	32	48	172	35	22	47	25	104
					5 mg/liter fish					
1	34	23	26	30	94	37	37	0	32	30
2	36	21	31	22	123	33	26	8	17	69
3	36	27	30	25	107	32	26	19	27	114
4	37	25	19	30	158	33	34	23	17	103
5	37	18	38	26	157	34	34	30	29	110
6	38	20	35	29	145	33	33	20	6	83
					11 mg/liter fish					
1	38	62	56	16	140	34	30	0	14	22
2	38	71	30	27	218	34	40	14	41	119
3	41	69	45	28	177	29	64	16	29	129
4	40	70	42	25	250	33	43	12	35	117
5	41	67	36	24	234	31	49	30	45	148
6	44	65	34	22	221	32	45	33	69	151

[a] Derived from the initial number in the population less the emigrants; unaccountable losses of fish in the system were spread over the appropriate periods of time.

in distribution among the three groups already noted (Fig. 8) and emphasize the consistently disproportionate representations of the groups in these three areas of the aquaria.

TERRITORIAL AND HIERARCHICAL ORGANIZATION

Coho established and defended territories, forming a territorial mosaic in both riffles and pools. Territories were defined more sharply in the riffles, where they were accompanied often by increased aggression. Fish invading the riffles typically pursued a zigzag course as they advanced upstream, violating the territories of resident coho. Residents attempted to drive out the invaders and were usually successful. Invading coho fled rapidly downstream into the pool unless a resident was displaced.

Fish in the riffles were territorial, each defending an area surrounding the feeding station and extending upstream. Aggressive interaction was followed by return to the feeding station, a particular location at the downstream end of the territory. Only a few fish in the pools defended territories, about 15 and 11%, respectively, in the left and right systems. For example, of 13 territorial coho in pool 1 of the left system on July 24, 11 were 11 mg/liter fish and two were 5 mg/liter fish (Fig. 9). In pool 2, eight of 16 coho defended territories, four were 11 mg/liter fish and four were 5 mg/liter fish. No territories were held in either pool by 3 mg/liter coho. In pool 1 of the right stream aquarium on July 24, 11 mg/liter coho defended five of seven territories, and one 5 mg/liter and one 3 mg/liter fish each defended the remaining two territories. Nearly all of pool 2 was defended by a single, medium-sized 11 mg/liter coho although three 3 mg/liter coho were present. The dominant fish patrolled the pool and attempted to drive the 3 mg/liter fish upstream into riffle 2.

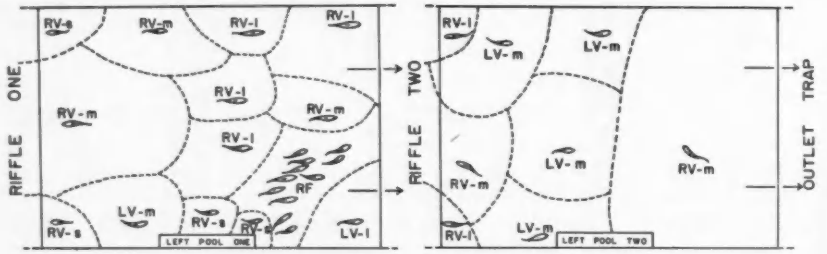

FIG. 9. Territoriality shown by population II in the pools of the left stream aquarium on July 24, 1964. Broken arrows indicate the direction of the primary current; RV = 11 mg/liter fish, LV = 5 mg/liter fish; *l* = large, *m* = medium, *s* = small; RF = resting fish.

Size of pool territory varied but, in general, larger fish defended larger territories in both pools and riffles. Downstream territories were often larger than upstream ones yet defending fish were often smaller than upstream defenders. For these reasons the average size of territories in riffle 2 and pool 2 would be misleading, but comparison of territory sizes in pool 1 and riffle 1 is of value. Coho defended larger territories in the riffles than in the pools: 0.17 and 0.10 m^2, respectively, in the left system; 0.22 and 0.16 m^2 in the right system. The larger mean size of coho in the right system may account for the larger territories.

Pool territoriality primarily involved orientation to the secondary current (Fig. 10, pool 2, side view), where coho on the bottom faced downstream (Fig. 9).

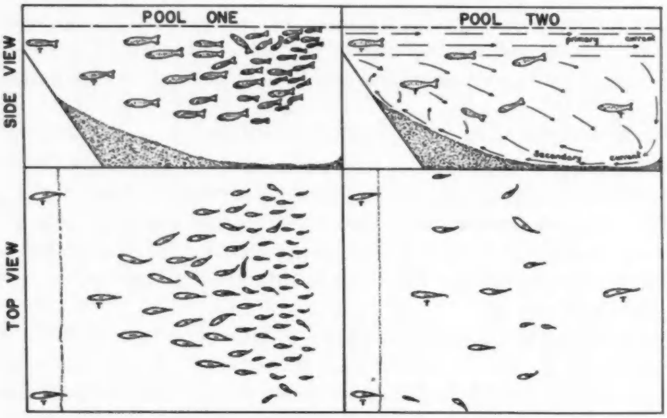

FIG. 10. Typical size hierarchy shown by population II in pools of the left stream aquarium. Based on detailed sketches and visual counts made between July 12 and July 26, 1964. T = territorial fish.

Most of the fish in the pools (70%) belonged to a size hierarchy orienting to the primary current flowing off the upstream riffle. This size hierarchy is represented diagrammatically (Fig. 10). In pool 2 of the left system, the hierarchy was recognizable but more loosely formed due to the small number of coho present. The foremost fish in these hierarchies were large, aggressive individuals, size decreasing downstream so that the trailing fish belonged predominantly to the 3 mg/liter group.

In addition to territorial and hierarchical social structure in pool 1 of the left aquarium, a third group of coho constituted a dense aggregation of large, nonterritorial individuals, usually less than 12, oriented in the secondary current at the bottom of the pool (Fig. 9). They were called "resting fish" since they exhibited very low aggression, little movement, and stayed close to each other. Of 11 such fish in pool 1 (about 75 coho were in the pool) of the left aquarium on July 24, five were 11 mg/liter fish, four were 5 mg/liter fish, and two were 3 mg/liter fish. Most of these coho had distended digestive tracts. Resumption of feeding by these fish was preceded by rising aggression directed at neighboring fish, particularly at territorial individuals in the pool, and was usually followed by invasion of riffle 1 and intensely aggressive interaction with territoryholders there. During 5 consecutive days of observations on social organization in pool 1 of the left aquarium, the 11 mg/liter coho dominated the positions at the riffle-pool boundary and no 3 mg/liter fish were observed here (Table 10). Most of the fish in the upstream half of the pool were 11 mg/liter fish and were either territorial fish or dominant individuals in the pool hierarchy. The 3 mg/liter coho counted in the front half of the pool are considered to be wanderers as they were chased off quickly. The upstream portion of the hierarchy was dominated by 5 mg/liter coho (71%) whereas 3 mg/liter coho constituted most of the fish in the downstream portion (61%). Coho in the secondary current (territorial and resting fish) were predominantly 11 and 5 mg/liter fish, the 3 mg/liter coho making up but 10% of the fish in this category.

Aggressive Behavior in the Stream Aquaria

Fish were more aggressive in the riffles than in the pools ($P < .05$) in population I but not in population II (Table 11). Mean frequency of aggression in population II was 5.4 compared with 4.1 acts/coho during 10 min in population I, the difference being almost significant at the 5% level. In both populations, there was no significant difference in frequency of aggression between the left and right systems.

Despite differences in mean frequency of aggression, the incidence of threat display remained stable in both populations. Mean threat values composed 32.6% (population I) and 33.9% (population II) of total behavior recorded. Differences among environments and populations were not statistically significant; however, incidence of threat display was slightly higher in the riffles than in the pools for both populations (Table 11).

In both populations, the nip categories involving more aggression were observed more frequently in the pools than in the riffles but the difference was significant ($P < .01$) only in population II. Incidence of nipping was lower in population II ($P < .01$), and the incidence of "drive toward" was much higher ($P < .01$) than in population I. Population II coho were generally larger than those in population I while in the stream aquaria and were more frequently aggressive.

Frequency of aggression, water temperature, and rate of emigration were tested for possible correlations. In population I, a significant correlation was found between water temperature and emigration, where emigration decreased with increased temperature ($F = 23.2$ with 1.6 df, $P < .01$). The corresponding regression for population II was negative but insignificant. The biological significance of such correlations remains obscure since other relevant variables, e.g. food supply, were not measured directly.

Diel Behavioral Rhythm

Significant diel changes in density of fish were recorded for riffle 1 only. In both stream aquaria, the numerical changes involved passage of coho to and from pool 1. For example, in the sequence A–E (Fig. 11), five daily density

TABLE 10. Distributions of coho fry on the basis of positions in pool 1 of the left aquarium, according to concentration of dissolved oxygen[a] prior to yolk absorption. Compiled from morning and afternoon counts made during 5 consecutive days.

		11 mg/liter			5 mg/liter			3 mg/liter		
Position in pool	Total no. of fish counted	No. of fish	Range	% of total	No. of fish	Range	% of total	No. of fish	Range	% of total
At boundary between pool 1 and riffle 1	28	24	2–3	85.7	4	0–1	14.3	0	0	0
In front half of pool, including runway[b]	54	36	2–5	66.7	15	1–2	27.8	3	0–1	5.6
In front portion of hierarchy in primary current	127	29	2–4	22.8	90	7–12	70.9	8	0–2	6.3
In rear portion of hierarchy in primary current	186	19	1–3	10.2	54	4–8	29.0	113	8–14	60.8
In secondary current	154	93	6–14	60.4	42	2–6	27.3	19	1–4	12.3

[a] The per cent contributions of the three groups of coho to the total number of fish in the aquarium during the 5-day test period were: 11 mg/liter coho, 41.4%; 5 mg/liter coho, 37.1%; 3 mg/liter coho, 21.6%. The total population was 116 fish, about 80 of which were in pool 1.

[b] The runway is the sloping board at the front end of the pool which leads up to the riffle.

TABLE 11. Relative frequencies of aggressive acts shown by coho fry in the two populations in both aquaria during the spring and summer months.

	Population I				Population II			
	Riffles		Pools		Riffles		Pools	
Act	Acts	%	Acts	%	Acts	%	Acts	%
Nip contact	47	9.0	24	12.8	27	4.6	32	5.5
Nip close	68	13.0	34	18.1	32	5.5	76	13.1
Nip toward	76	14.5	29	15.4	31	5.3	88	15.2
Drive toward	154	29.4	43	22.9	284	48.6	190	32.8
Sum	345	66.0	130	69.1	374	64.0	386	66.6
Threat display								
Horizontal	148	28.3	47	25.0	130	22.3	124	21.4
Head up	None		5	2.7	None		39	6.7
Head down	30	5.7	6	3.2	80	13.7	31	5.3
Sum	178	34.0	58	30.9	210	36.0	194	33.4
Total acts	523		188		584		580	
Mean frequency of aggression[a]	7.0		2.5		5.4		5.4	

[a] Acts/coho during 10 min.

rhythms in riffle 1 are shown which consisted of high densities during the presunrise and post-sunset periods separated by fairly consistent periods of low density during most of the daylight hours. This density cycle occurred regardless of weather conditions and water temperatures, the weakest and strongest cycles (C and E) obtaining during similar weather conditions and course of water temperature (Table 12). The cycle was defined well when associated with a very shallow daily pulse in water temperature on June 30 (B). No daily changes in vertical distribution were discernable in the pools.

Coho in the stream aquaria fed actively at very low light levels. Infrared observations (Detectirscope, model 5500C — Varo, Inc.) and data derived by use of sliding screen partitions to isolate pools from riffles indicated that the high densities at dawn and,dusk in riffle 1 were probably sustained through the night. The disproportionate representation of larger fish in the riffle was not modified by increased density. The additional coho leading to increased densities at dawn and dusk came from pool 1.

Frequency of aggression and of invasion and retreat activity were measured from dawn to dusk to determine if the daily cycle of density change in riffle 1 involved competition for territorial rights in the riffle. Invasion and retreat activity were recorded at the boundary between the pool and riffle as the number of coho that invaded or fled from the riffle during 15-min observation periods made hourly throughout the day. Aggressive behavior was measured as the number of aggressive acts committed by individual fish (9–24), each observed for 30-sec intervals during 15-min observation periods.

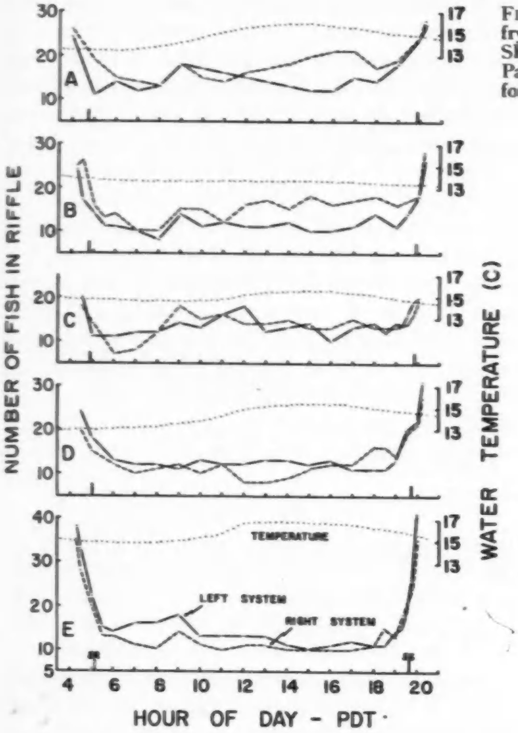

FIG. 11. Diel cycle in density of coho fry in riffle 1 of both stream aquaria. SR = sunrise, SS = sunset, PDT = Pacific Daylight Time. See Table 12 for dates and weather data describing sequence A through E.

TABLE 12. General weather conditions on days when activity cycles were recorded for under-yearling coho in population II.

	Date	Weather conditions
A	June 23	Completely overcast throughout day
B	June 30	Completely overcast until noon, clearing to a nearly cloudless sky by late afternoon
C	July 7	Cloudless and warm
D	July 15	Light rain until 0800 hr with partial clearing and occasional sun until 1300 hr, followed by a complete overcast for the remainder of the day
E	July 28	Cloudless and warm

The dusk and dawn peaks in density of fish in riffle 1 were accompanied by increased aggression and frequent attempts by coho invading the riffle from pool 1 to remain in the riffle and compete for food and space with residents (Fig. 12). Frequency of aggression showed a similar daily rhythm in the front half of pool 1, and involved interaction primarily between territorial fish in the pool and those invading the riffle.

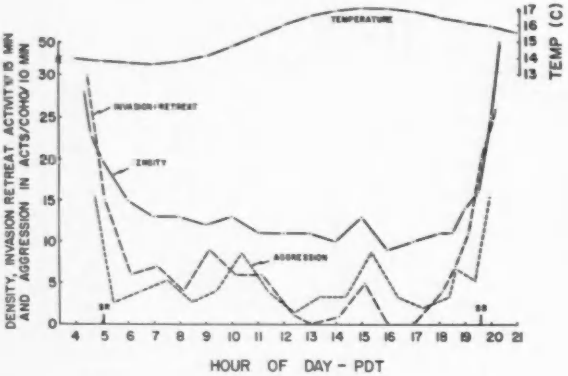

FIG. 12. Diel cycle of activity of coho fry involving competition for territory in riffle 1 of the left stream aquarium of July 24, 1964. Abbreviations as in Fig. 11.

GENERAL DISCUSSION

Embryos and fry exposed to average dissolved oxygen concentrations of 5 and 3 mg/liter were smaller at times of hatching and emergence, and underwent higher mortality during development than embryos and fry of the same parents exposed to 11 mg/liter and a slightly lower temperature. Most mortality occurred prior to hatching.

Smaller size of the 5 and 3 mg/liter fish at time of hatching and emergence was primarily the outcome of reduced oxygen supply. Probably these decreases in size were made conservative by the minor differences in developmental temperature. Hayes and Pelluet (1945) found that increased developmental temperature increased the efficiency of conversion of yolk to embryo, and, from their data, the increase in conversion efficiency in the present experiment would have amounted to 1.3% for the 5 mg/liter fry and 3.0% for the 3 mg/liter fry.

In the left stream aquarium, competition for the food supply, composed chiefly of incoming drift, was expressed clearly in growth rate, number of resident fish, and net production, and reflected the degree of hypoxial stress imposed prior to yolk absorption. Competition for food was much reduced in the tank where a hatchery diet (in excess until late in the experiment), combined with high population density and a much simplified environment, discouraged territorial and hierarchical behavior.

In both populations, emigrants from the left system that were transferred to the right one remained there. At the end of the experiment, these fish were as large (11 mg/liter coho) or larger (5 and 3 mg/liter coho) than fish remaining in the left system and they showed similar or higher cœfficients of condition. Larger size was the outcome of increased growth rate after emigration and was due to less competition for food and space in the right system. Instantaneous growth rates indicate that fish leaving the left aquarium were smaller than those remaining, the difference in size being greatest in the fry most stressed prior to yolk absorption. When transferred to the right system, the 3 mg/liter fish whose growth had been most severely suppressed prior to emigration showed the most marked increase of instantaneous growth rate.

In the left aquarium, the ecological advantage conferred on the fry reared at 11 mg/liter of dissolved oxygen prior to yolk absorption was mainly the outcome of behavioral interaction whereby dominance associated with larger size determined the feeding relations within the population. Larger coho retained better feeding conditions than did smaller fish by defending those areas where the drift was most abundant, namely, the upper riffle and the front of the pool immediately following it. In both aquaria, the gradients in numbers and in size of individual fish reflected the importance of the incoming drift in distributing fish in the systems.

Juvenile coho in streams rely chiefly on invertebrate drift, a food supply that is spatially diluted and probably limited. They exhibit food-related, conventionalized competition for space through territoriality. The present results also show that they may form size hierarchies in pools that are secondarily related to space and probably adaptive during periods of abundant food supply.

Survival to emergence marked the onset of a stream life attuned to social relations in these aquarium populations maintaining volitional residence. In small hierarchies confined in aquaria, higher survival, resistance to disease and starvation, better growth and reproductive opportunity were benefits accrued from high social position (Allee et al., 1948; Barrow, 1955; Magnuson, 1962; Yamagishi, 1962).

Among coho fry emerging in natural streams, size is probably of major importance in determining the pattern of food distribution. Although the quantity of yolk in the egg is the major determinant of potential size of salmonid fry at emergence (Gray, 1928), both dissolved oxygen concentration and water temperature may reduce potential size extensively, and lengthen the period of time spent in the redd. The influence of temperature in this regard was pointed out by Hobbs (1953). The present results show that small fry are poor competitors and face almost certain death from predation, disease, or starvation (most likely a combination of these) unless competitive stress resulting in poor growth is relieved by emigration. Smaller size resulting from poor growth no doubt increases the probability of predation in natural environments since small fry spend more time within a size range most vulnerable to predation (Larkin, 1956).

Decreased size of fry at time of emergence due to reduction of growth efficiency may not be the only immediate outcome of nonlethal hypoxial stress. Possibly, subtle differences in vitality or impairment of specific physiological functions may accompany reduction in size. But, if the present ecological findings are associated primarily with size relations within the population, one may assume tentatively that environmental factors leading to reduction in size of fry at emergence are most likely deleterious to such fry in natural populations. Thus, efforts to establish dissolved oxygen standards at some concentration, or range of concentrations, below saturation and supposedly advantageous to natural populations of salmonids are questionable bionomically, especially when such standards are thought desirable for upriver regions important as spawning and rearing areas.

ACKNOWLEDGMENTS

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Influence of Temperature Change on Spontaneous Locomotor Activity and Oxygen Consumption of Atlantic Salmon, *Salmo salar*, Acclimated to Two Temperatures

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PETERSON, R. H., AND J. M. ANDERSON. 1969. *Influence of temperature change on spontaneous locomotor activity and oxygen consumption of Atlantic salmon, Salmo salar, acclimated to two temperatures.* J. Fish. Res. Bd. Canada 26: 93–109.

Measurements of the effect of rapid change in temperature on the spontaneous activity and oxygen consumption were made on Atlantic salmon underyearlings acclimated to 6 or 18 C. The new levels of imposed temperature ranged from 6 to 30 C for both acclimations. At similar test temperatures the calculated standard metabolic rate of the fish acclimated to 6 C was higher than that of the fish acclimated to 18 C, up to about 23 C, where the two curves relating oxygen consumption and temperature intersect. Spontaneous activity could be separated into two phases, a transient phase occurring during the actual period of temperature change, and a stabilized phase. The transient phase was characterized by a peak in activity which was found to be correlated with the rate, rather than the amount, of the temperature change. In general, the peak was higher for fish acclimated to 18 C. The relation between activity in the stabilized phase and test temperature was characterized by a plateau or maximum in the general region of the previously determined selected temperature. Except at the coldest test temperature (6 C), the fish acclimated to 6 C were more active in the stabilized phase than were the fish acclimated to 18 C at similar test temperatures. Complete acclimation for both metabolism and activity, between 6 and 18 C, requires about 2 weeks, regardless of the direction of the temperature change.

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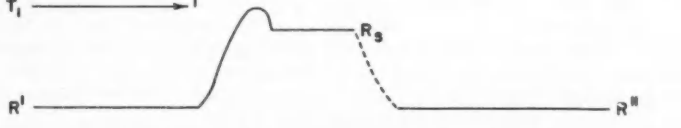
INTRODUCTION

WHEN AN ORGANISM is subjected to a rapid temperature change, a characteristic sequence of change usually occurs in any temperature-dependent function. This pattern of change is illustrated below (modified from Prosser, 1965).



R' represents the level of activity of the rate function prior to temperature increase to T_2. The level immediately rises, goes through a transient overshoot,

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then levels off at a value somewhat higher than R′. This level is termed the "stabilized level" (R_s). The overshoot period usually ends after a few minutes, whereas R_s may endure for several hours. With time, homeostatic processes tend to return the function to its original level (R′) — at which time the organism is said to be acclimated. Several days, or even several months, may pass before the fully acclimated state (R″) is attained. Compensation may be complete (in which case R″ = R′), but is often only partial. If the temperature were decreased, rather than raised, the expected sequence of events would be the mirror image of the above diagram (time representing the axis of symmetry).

One may vary T_2 in the above diagram, measure the stabilized level of a rate function at each of those temperatures, and plot the values against their respective test temperatures. The result is termed an "acutely" measured curve. In the experiments reported here, the overshoot and stabilized levels of spontaneous locomotor activity of juvenile Atlantic salmon, *Salmo salar* (Linn.), were studied for a number of different temperature changes. Rates of oxygen consumption were measured in the stabilized state. The length of time required to establish a new acclimation state was also studied.

MATERIALS AND METHODS

ORGANISMS

The Atlantic salmon used were underyearlings and were obtained from the Canada Department of Fisheries hatchery at Saint John, N.B., in September 1965 and 1966. They were maintained under conditions of continuous running water with constant aeration. Their food was a daily administration of minced, raw beef liver, mixed with a commercial pellet food. The fish were maintained at one of two acclimation temperatures (18.0 ± 0.5 C or 6.0 ± 0.5 C) at least 1 month before experimental use. The daily light regime was 12 hr light–12 hr darkness with the light period beginning at 8:30 AM. Fish were placed in the experimental apparatus 12 hr before experimentation and had been starved 36 hr when tested. All fish were weighed after use.

APPARATUS

The experimental apparatus (Fig. 1) consisted of an outer, rectangular box (G) of 6.0-liter capacity, which housed an annular chamber of 2.3 liters (H). The entire assembly was inside a plywood cabinet, painted black inside.

The water of the inner chamber was in contact with that of the outer via a ¼ inch inside diam piece of plexiglass tubing, 1 inch long. This permitted introduction of water into the inner chamber. A similar, diametrically opposed piece of tubing led via a length of tygon tubing to the sampling bottles.

The central "hole" of the annular chamber (J) enclosed a 1.5-w bulb that activated four photocells. The photocells were set in housings (A) at 90°-arcs around the outer circumference of the annulus. The photocells received light from slits in the otherwise blackened central portion of the annulus. Thus, one complete circuit of the chamber resulted in one unit of activity recorded by each of the four photocells. The top of the annulus contained two watertight ports — one for adding the fish (B), the other for inserting a thermometer (C).

All experiments were conducted in a constant temperature room maintained at the test temperature.

EXPERIMENTAL PROCEDURE

Prior to each determination of oxygen consumption, the annular chamber was flushed at the rate of 1 liter/min for 20 min. Just before flushing was discontinued, a sample was taken to measure the initial oxygen concentration in the annulus. The flow of water was then shut off for a period depending upon the temperature. At the end of the shutoff period, a sample was taken by

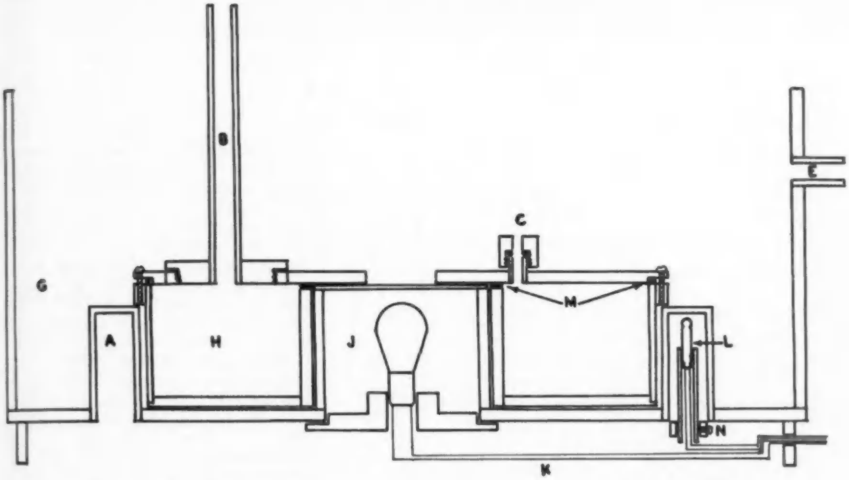

FIG. 1. Side view of respirometer: (A) photocell housing; (B) feeding and fish entry port; (C) thermistor port; (D) inlet tube into inner chamber; (E) overflow for outer chamber; (F) outlet tube from inner chamber; (G) outer chamber; (H) inner chamber; (J) housing for activator lamp; (K) leads to variac; (L) photocell; (M) rubber gaskets; (N) screw for adjustment of photocell height.

siphoning the water off at 25 ml/min until a 250-ml sample was obtained. The first 50 ml were discarded to eliminate the water present in the tubing during the experiment. During the total test time, the fish rarely lowered the oxygen concentration by more than 2 ppm. The error due to mixing of water entering the annulus during sample taking was estimated to be 5% and was not corrected for.

The oxygen content of the water was determined by the unmodified Winkler procedure. A 200-ml sample was titrated amperometrically in a mono-metallic platinum+ platinum− electrode system which gave a very sharp voltage drop (0.2 v) at the end point.

The test temperature was changed by heating or cooling the water in the reservoir (not shown in Fig. 1) feeding the outer chamber as desired (by adding hot water or ice), flushing the outer chamber rapidly, and then flushing the inner chamber with the water at the new temperature.

After each experiment, the fish was returned to its acclimation temperature and was not used again for at least 1 week.

RESULTS

GENERAL

Most of the results are based upon measurements of the locomotor activity and oxygen consumption made 2 hr before and 6 hr after an abrupt change in environmental temperature. The initial temperature was always the acclimation temperature. Typical results are shown for individual fish acclimated to 6 C (Fig. 2). The time-course of the temperature change is indicated by half-solid circles (upper left curve in each panel). Activity (open circles) is given as pen deflections per hour. It was possible to measure activity, but not oxygen consumption (solid circles), during the period of temperature change. Specific

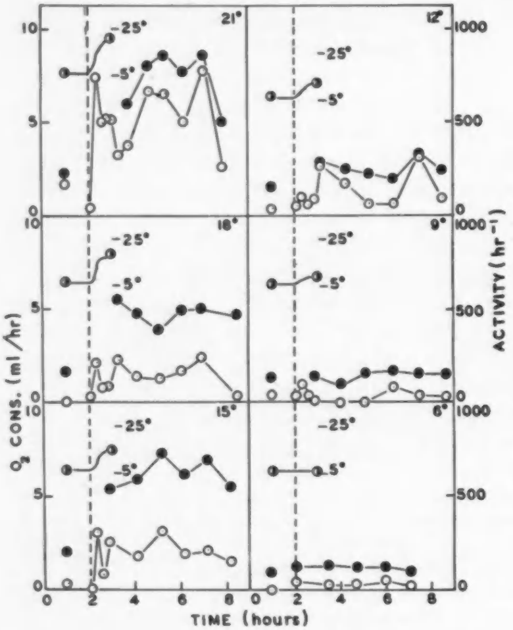

FIG. 2. Representative experiments performed with salmon acclimated to 6 C at various test temperatures (shown in upper right-hand corner of each panel). Solid circles represent oxygen consumption, half-solid circles show the pattern of temperature change from acclimation temperature to test temperature, and open circles represent "spontaneous activity." Vertical broken lines indicate the time when temperature change was initiated. Activity units are given on the right ordinate, oxygen consumption units on the left. Each experiment shown is one of nine similar experiments. The insets (25° and 5°) are temperature scales to which the lines enclosed by the half-solid circles are to be referred.

reference to one of the six experiments (viz., top left panel, temperature change 6–21 C) will assist in further description of the experimental protocol.

The vertical line drawn at the 2-hr ordinate denotes the time when temperature change was begun. Initially the temperature increases slowly. This coincides with the time required to flush out the outer chamber. The rapid temperature change, followed by the decrease in rate of change as the final steady-state temperature is reached, corresponds to the flushing of the annulus. The time required to change the temperature depends upon the time required to flush the chambers rather than on the magnitude of the temperature change. Thus the times are the same for all experiments — about 30 min. Activity was relatively low prior to the temperature change, but increased rapidly to a peak as the temperature was raised. After the temperature had stabilized, the activity remained high, although variable. As would be expected, oxygen consumption and activity show a direct correlation.

For the fish acclimated to 18 C, a series of experiments (similar to those in Fig. 2) was conducted, except that in this case it was possible to both increase and decrease the environmental temperature. For each temperature change, in both acclimation series, a minimum of nine fish was used. In all, 135 separate experiments were conducted. Analyses of the results follow.

ACTIVITY AND OXYGEN CONSUMPTION

Activity, as a prime user of energy, obviously will have a marked effect on oxygen consumption. In order to eliminate the influence on oxygen consumption, it is conventional to calculate the "standard" oxygen consumption (Beamish, 1964), which is the rate of oxygen consumption on a unit weight basis at zero activity. Although the standard oxygen consumption so determined has little ecological significance, it nevertheless provides a useful reference base.

A reliable estimate of the regression of oxygen consumption on activity $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ depends upon a sufficient difference in range of spontaneous activity. This variation frequently failed to occur in these experiments. To overcome the difficulty, it was necessary to obtain estimated values of $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ for fish of different weights, so that these values could be used in those experiments where the activity was not sufficiently variable. The data from all 135 experiments were grouped into six weight-classes (grams) (0–10, 10.5–20, 20.5–30, 30.5–40, 40.5–50, and 50.5–60). The mean value (weighted for degree of variation in activity) for the $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ for each weight-class was then calculated. The justification for this method of calculating the mean lies in the assumption that the greater the variation in activity, the more reliable the estimate of oxygen requirement for any level of activity, including zero activity. In Fig. 3 the weighted means of the $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ values for the fish in the various weight-classes are plotted against the mean fish weight in each class. Most of the fish used lay within the 10–40-g weight range, and for this range the points agree quite well with the calculated line.

The standard oxygen consumption was then calculated from the formula $a = y - bx$ (where y = average observed oxygen consumption, b = the value of $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ obtained from the line in Fig. 3 for a fish of the appropriate weight, x = the average observed activity). It should be noted that the validity of Fig. 3 depends upon several assumptions. The analysis assumes that $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ is independent of both acclimation and test temperature for fish of any given weight. It also depends upon the absolute measurement of activity by the photocells being independent of fish size. It should also be noted that Brett (1965) observed that maximum swimming speed in the Pacific salmon (*Oncorhynchus nerka*) was not proportional to body size, in spite of the relative increase in scope for activity. This would imply that $\frac{\Delta O_2\text{consump.}}{\Delta\text{ act.}}$ as a function of body weight, might not be linear. However, this may only apply at swimming speeds near maximum; speeds of this sort were not attained in these experiments. Also, increased activity here does not necessarily imply a greater swimming speed, but merely a greater number of movements.

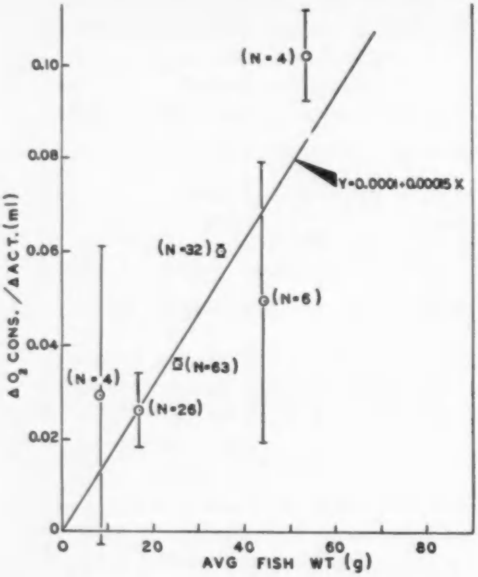

FIG. 3. ΔO_2 consumption/Δactivity plotted as a function of fish weight. N = the numbers of fish represented by each point. Vertical lines indicate ± one standard error.

FISH WEIGHT AND STANDARD OXYGEN CONSUMPTION

The metabolic rates of all fish were corrected to a common weight of 25 g by the use of a correction factor obtained from the usual equation relating standard metabolic rate to body weight, $R = AW^b$, where R = metabolic rate, W = body weight, and A and b are constants. The pooled value of "b" for all fish used was 0.59 ± 0.08. This is somewhat lower than the values usually obtained for fish ($\simeq$ 0.8). Brett (1965) obtained a value of 0.62 for juvenile Pacific salmon, so it is possible that juvenile salmon are characterized by lower "b" values than are fish generally. No statistically significant effect of test temperature or acclimation temperature on the "b" value could be distinguished, possibly because of the small numbers (nine) of fish used for each combination of test temperature and acclimation temperature.

ACCLIMATION TEMPERATURE AND OXYGEN CONSUMPTION

The standard rates of oxygen consumption, corrected to a common 25-g weight, are shown in Fig. 4 at the various test temperatures for fish acclimated to 6 or 18 C. The curve for the fish acclimated to 6 C is rotated clockwise and translated to the left when compared with that for the fish acclimated to 18 C (Type IVA adaptation) (Prosser and Brown, 1961). The regression lines for the two acclimations tend to converge at about 23 C. The Q_{10}'s are 2.2 and 3.0 for the fish acclimated to 6 or 18 C, respectively. The increase in standard rate of oxygen consumption at 6 C for fish acclimated to 6 C is 56% greater than the consumption at 6 C for fish acclimated to 18 C. The degree of compensation, however, is only about 20%.

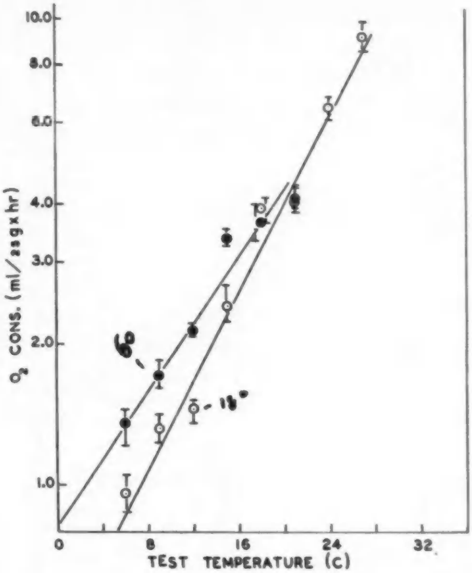

FIG. 4. Standard oxygen consumption of salmon acclimated to 6 or 18 C at various test temperatures. Closed circles show values for acclimation to 6 C, open circles for acclimation to 18 C. Vertical bars indicate ± one standard error.

ACTIVITY DURING TEMPERATURE CHANGE

The average values for the activity of all fish tested have been calculated for temperatures other than that to which the fish were acclimated. This is shown in Fig. 5 for acclimation to 18 C and in Fig. 6 for acclimation to 6 C (open circles, activity; closed circles, temperature change). The final value of the line describing these data (broken line on the right-hand end of each line) corresponds to the average value for the activity during the stabilized period.

For each acclimation series, the initial activity is approximately the same (because in each experiment the initial temperature is always the acclimation temperature). This initial level is about 40 units/hr for the fish acclimated to 6 C and about 150 units/hr for the fish acclimated to 18 C.

When the temperature changes, it is apparent that for fish of both acclimations — regardless of the direction of the temperature change — the activity first increases and goes through an overshoot. This response is not too well defined for the salmon acclimated to 6 C when the temperature changes are relatively small (6–9 C; 6–12 C; 6–15 C). However, in these instances the activity does not rise smoothly to its final level during the stabilized phase. Instead, there is a small peak or plateau interrupting the rise in activity. This has been interpreted as being equivalent to the overshoot activity so evident in the other experiments.

Figures 5 and 6 reveal one final point of interest. Comparison of the maximum level of activity attained for each temperature change with the magnitude of the temperature change suggests a simple relation — the greater

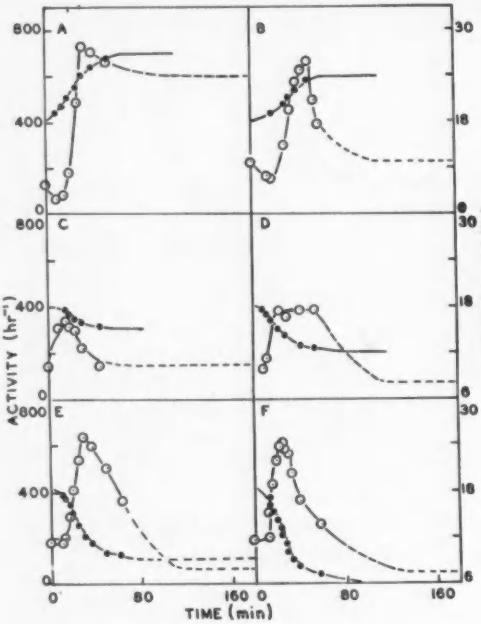

FIG. 5. The pattern of activity with changing temperature for fish acclimated to 18 C: (A) 18 C → 27 C; (B) 18 C → 24 C; (C) 18 C → 15 C; (D) 18 C → 12 C; (E) 18 C → 9 C; (F) 18 C → 6 C. Open circles represent activity; solid circles represent temperature. Each panel is an average of nine similar experiments. Activity units are recorded on the left-hand ordinate, temperature on the right. The horizontal broken lines are the mean levels of activity obtained in the stabilized state. These have been extrapolated from the final measurement of activity taken during the period of temperature change.

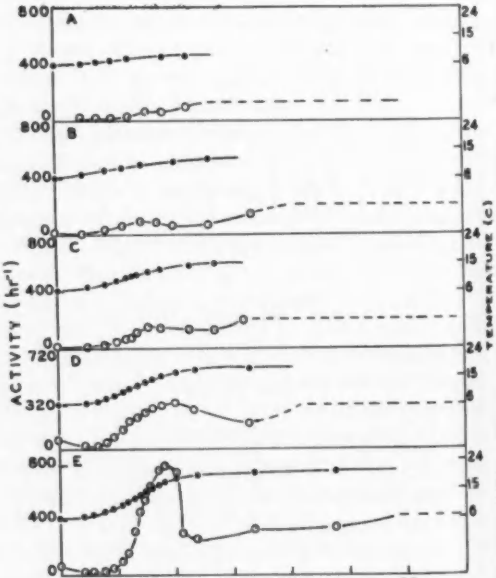

FIG. 6. The pattern of activity with changing temperature for fish acclimated to 6 C: (A) 6 C → 9 C; (B) 6 C → 12 C; (C) 6 C → 15 C; (D) 6 C → 18 C; (E) 6 C → 21 C. Other aspects similar to Fig. 5.

the temperature change, irrespective of the direction of the change, the greater the peak activity achieved. This is investigated further in Fig. 7, which shows the relation between peak activities and the amount by which the temperature change was less or greater than the initial temperature, the latter denoted by "zero temperature change". The data for acclimation to 18 C (Fig. 7B) indicate that a strong positive correlation exists between the absolute amount of temperature change and the height of the activity peaks. The response is somewhat stronger when the temperature is increasing than when it is decreasing, as suggested by the greater slope associated with temperature increase (right-hand curve). A positive correlation also holds for fish acclimated to 6 C for increases in temperature (Fig. 7A). (No experiments were performed below 6 C, because lower temperatures could not be maintained.) It will be noted that over a comparable temperature range, the response of the warm-acclimated salmon was appreciably more vigorous than that of the cold-acclimated fish.

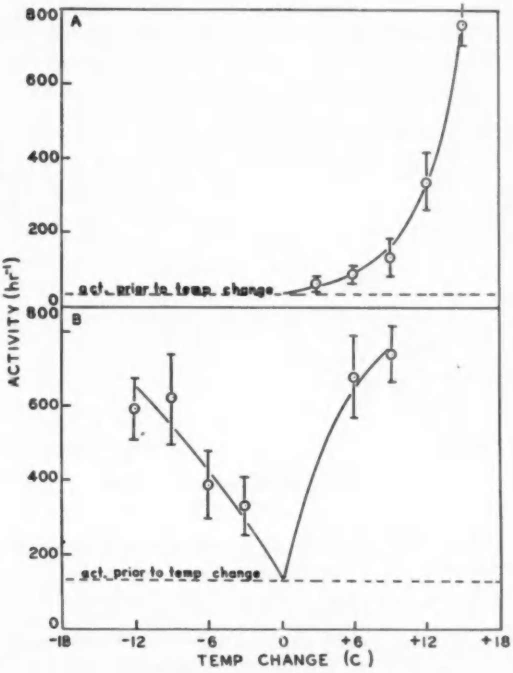

FIG. 7. Peak activity associated with the period of temperature change: (A) fish acclimated to 6 C; (B) fish acclimated to 18 C. Vertical bars represent ± one standard error.

However, although temperature change and activity are clearly related in these experiments, the causative link is not necessarily temperature change per se. The choice of *magnitude* of temperature change as the independent correlate was somewhat arbitrary since the time for completion of temperature change was the same in all cases. This means that the *rate* of temperature change also varied between experiments; and concomitantly, and directly, with the magnitude of the temperature change.

To determine which concomitant attribute of the temperature change (rate or degree) is important, experiments were performed in which the absolute temperature change was held constant (18 C → 12 C) and the rate of change was varied. This was achieved by varying the flow rate through the chamber when changing the temperature. As the rate of temperature change is increased, the height of the activity peak increases, then shows a tendency to level off between 600 and 800 units/hr (Fig. 8). This is very near the activity level where the curves for fish acclimated to 18 C levelled off in Fig. 7. The value of activity obtained for lowering of the temperature from 18 to 12 C (Fig. 7) was about 400 units/hr. Since about 1 hr was required to complete the temperature change, the rate of change would be about 0.1 degree C/min. A value of 400 units/hr for a rate of change of 0.1 degree C/min is very near the value predicted by the curve in Fig. 8. Therefore, the two sets of results appear to conform, if rate of temperature change is the basis of comparison. Evidently, then, it is *rate* of temperature change, not the amount of change per se, which affects the level of spontaneous locomotor activity. However, the reciprocal experiment, holding the rate of temperature change constant, and varying the absolute amount of change, was not performed, because of limitations set by the apparatus.

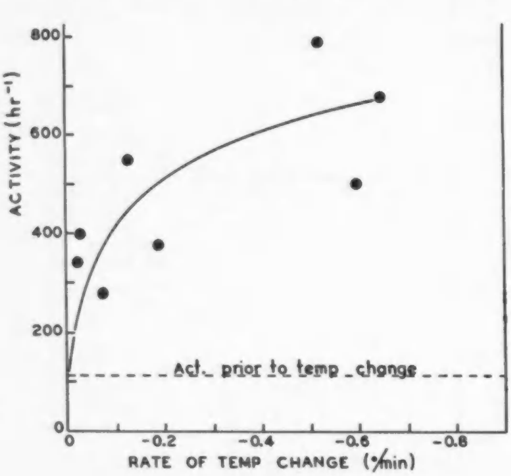

FIG. 8. Peak activity for different rates of temperature change. Absolute amount of change held constant (18 C → 12 C). Acclimation temperature was 18 C. Each point is the average of three experiments.

ACTIVITY IN THE STABILIZED STATE

In Fig. 9, the activity measured during the stabilized state (1–6 hr after the change in temperature) is shown. The rate of activity increases sharply with increasing temperature until a maximum is reached at about 12 C for

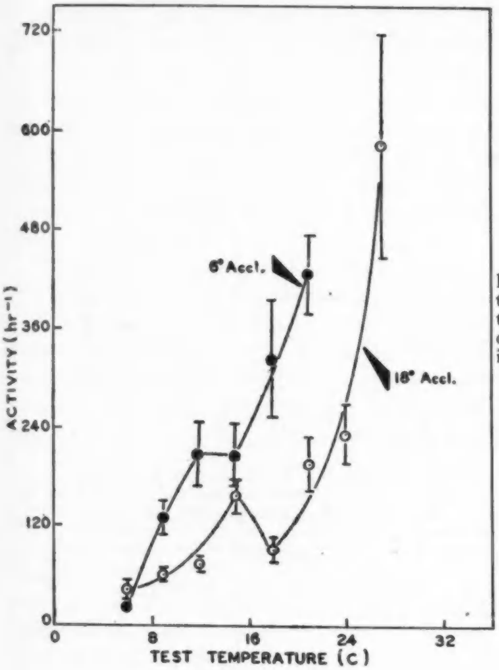

FIG. 9. Spontaneous activity during the "stabilized phase" at various test temperatures. Each point is an average of nine experiments. The vertical bars indicate ± one standard error.

salmon acclimated to 6 C and at 15 C for salmon acclimated to 18 C. After these peaks the activity either levels off or decreases for a brief temperature range, then increases sharply again. In general, the activity tends to be higher for the cold-acclimated fish with the curves converging near 6 C. It is obvious that, for both acclimations, the spontaneous activity at very low temperatures is greatly reduced. Even when acclimated to 6 C, salmon are extremely sluggish at this temperature.

LONG-TERM RESPONSES TO TEMPERATURE CHANGE

Thus far only the short-term responses of the salmon to temperature change have been considered. The long-term changes, during which the spontaneous activity and standard oxygen consumption characteristic of the stabilized state at one acclimation grade into those characteristic of another acclimation, remain to be considered. The time course of these changes, including the range of values recorded, are shown in Fig. 10 and 11 for 18 C → 6 C change in acclimation, and the reverse procedure, respectively. Three fish were tested for each procedure. Average fish weights are also shown. The initial values shown in each figure are for fish acclimated at least 1 month at the initial acclimation temperature.

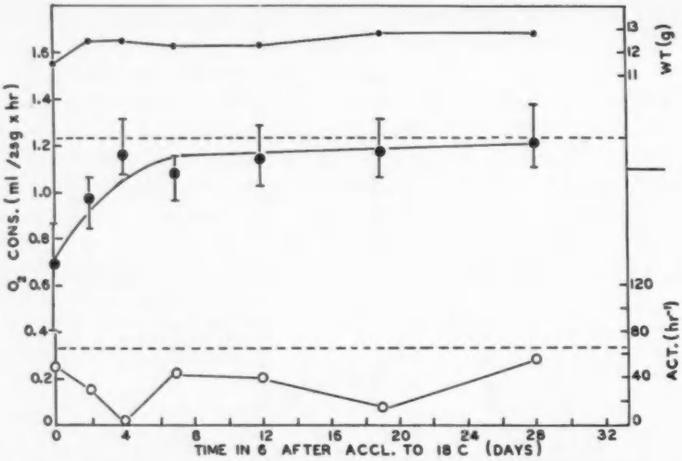

FIG. 10. Effect of prolonged decrease (18 C → 6 C) in temperature on weight (small solid circles), oxygen consumption (large solid circles), and activity (large open circles). Each point is the average of values obtained from three fish (same fish used throughout). Broken lines refer to oxygen consumption (*upper line*) and activity (*lower line*) of fish fully acclimated to 6 C, based on averages of nine different fish which had been held at the acclimation temperature for at least 2 months. Vertical bars represent the range.

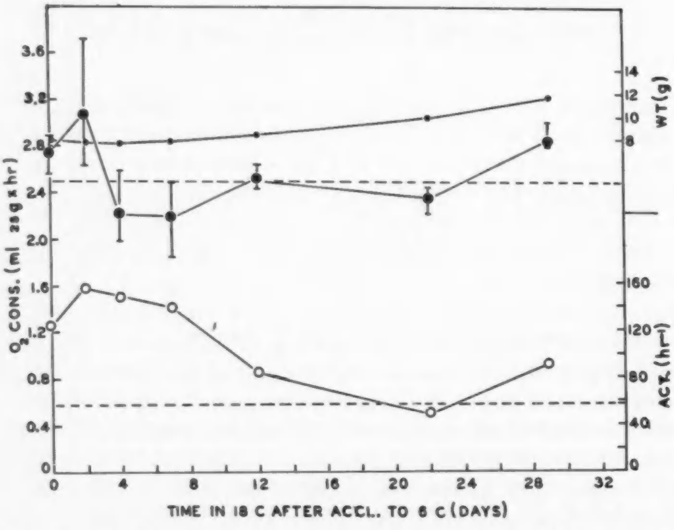

FIG. 11. Effect of prolonged increase in temperature (6 C → 18 C) on weight (small solid circles), oxygen consumption (large solid circles), and activity (large open circles). Each point is the average obtained from three fish (same three fish used throughout). Broken lines refer to oxygen consumption (*upper line*) and activity (*lower line*) levels of fish fully acclimated to 18 C, based on the average of nine separate fish which had been held at the acclimation temperature for at least 2 months. Vertical bars represent the range.

For the fish acclimated at 18 C, tested at various intervals after being placed in 6 C water (Fig. 10), the oxygen consumption increases rapidly during the first 5 days or so, and then the rate of increase falls off sharply. The acclimatory process is over by about the second week (Fig. 10). This is supported by the fact that the mean standard oxygen consumption of nine different fish maintained at 6 C for at least 2 months, is virtually the same (1.24 ml $O_2/25$ g per hr; broken line, Fig. 10), as that measured at the 12th, 19th, and 28th days of the experiment. About 80–90% of the metabolic acclimation is apparently complete within the 1st week.

The activity drops markedly during the first 3 or 4 days in 6 C. This was also observed in fish kept in our holding tanks under similar conditions. After 6 days, although there was some variation, the general impression given is that the activity remained more or less constant at a level characteristic of the fully acclimated state. (The mean activity of the nine fish referred to above was about 65 units/hr; broken line, Fig. 10). Acclimation of activity therefore appears to have been completed in about 2 weeks or less.

Weight increased abruptly after the fish were exposed to the low temperature. The weight averaged 10% higher after 2 days at 6 C than it was before the temperature change.

An investigation of the rate of acclimation from 6 to 18 C presented some problems. As previously noted (Fig. 4), the standard oxygen consumption curves for fish acclimated to 6 or 18 C intersect at about 23 C. Therefore, the standard oxygen consumption of fish acclimated to 6 C would differ only slightly from that of fish acclimated to 18 C when both rates are measured at 18 C. Neglecting the possbility of transient changes, the expected change in standard oxygen consumption when a fish acclimated to 6 C is put into 18 C, would be a relatively small decrease. One way to avoid the problem would have been to drop the fish down to a lower temperature periodically for measurement of oxygen consumption. This frequent return to a lower temperature for substantial periods of time could conceivably affect the acclimation process; and in any event, it introduces another variable. Therefore, it was decided to measure the oxygen consumption at 18 C after various lengths of time in 18 C.

Initially the standard oxygen consumption (Fig. 11) was slightly above the level characteristic of fish fully acclimated to 18 C — as would be expected. On the 2nd day the oxygen consumption was slightly higher, then dropped below the level for acclimation to 18 C on the 4th and 6th days. A partial return from this low level occurred between the 6th and 12th days when the oxygen consumption appeared to reach its final value. Again acclimation seems to have taken about 2 weeks or less. However, unlike the case for coldwards acclimation (Fig. 10), the time-course for warmwards acclimation appears to exhibit both an initial overshoot, and an undershoot before the final acclimated condition is reached.

Activity appears to go through an initial overshoot phase before reaching, in about 2 weeks, the lower value characteristic of the fully acclimated condition. The fish lost weight up to the 6th day, but thereafter weight increased steadily.

It is perhaps worth noting that the values of the fully acclimated fish shown in Fig. 10 and 11 as broken lines do not always correspond exactly to the values given previously. This is because the work on long-term responses to temperature change was done a year later than the previous work and thus the nine "control" fish were of a different year-class. Any discrepancies, however, appear to fall well within the limits of standard errors.

DISCUSSION

EFFECT OF TEMPERATURE ON O_2 CONSUMPTION

As shown, the relation between standard oxygen consumption and temperature for the salmon acclimated to 6 C differs from that for salmon acclimated to 18 C in two respects. The rates are higher for the cold-acclimated fish at comparable temperatures, and the Q_{10} is less for the cold-acclimated fish, i.e., the curve for cold-acclimated fish is translated to the left and rotated clockwise with respect to the curve for salmon acclimated to 18 C. Similar effects of acclimation to a lower temperature have been reported previously in the literature. Precht (1951) performed similar experiments with eels (*Anguilla vulgaris*) acclimated to 11 and 26 C. The relationship between the curves for the two acclimations are similar to those obtained for salmon, with an intersection temperature of 21 C. Precht (1951) obtained 30% compensation as compared with 20% for salmon. The Q_{10} values for eels acclimated to 11 or 26 C were 1.5 and 4.0, respectively. The respiratory rates were much lower for eels than for salmon at comparable temperatures, as might be expected, since the eel is a more sluggish animal. Grigg (1965) also obtained similar results with his investigation of the Australian lungfish (*Neoceratodus*), with the "cold" (18 C) and "warm" (25 C) acclimation curves intersecting at about 35 C. Compensation appeared to be nearly complete for the lungfish. The rates of oxygen consumption for the lungfish are rather low, being roughly $\frac{1}{3}$ that for eels and $\frac{1}{12}$ that for salmon (when compared at a common temperature of 18 C). Neither Grigg (1965) nor Precht (1951) measured activity; however, they presumably maintained the fish under conditions of minimal activity.

It has been suggested (Prosser, 1965) that a change in slope (rotation) of any rate function (e.g. O_2' consumption) with change in acclimation implies some qualitative change in the nature of some enzyme, or a change in metabolic pathways. The basis for this view is that a quantitative change in one or more enzymes would be unlikely to alter the Q_{10}, but would rather result in an increase (or decrease) in the rate proportionately at all temperatures (simple translation). Hochachka (1967) was able to show qualitative changes in lactic dehydrogenase in fish with change in acclimation temperature, since new isozyme subunits appear. Also, Ushakov (1967) demonstrated changes in the temperature at which denaturation occurs for various fish proteins after change in temperature. Our results, showing both rotation and translation, suggest that both qualitative and quantitative changes occur in the metabolic enzymes of salmon.

ACTIVITY DURING TEMPERATURE CHANGE

That both increase *and* decrease in temperature results in an overshoot in activity is noteworthy. This does not conform with the general pattern presented in the introduction, where it was implied that a decrease in temperature would result in an undershoot. The finding, however, can be easily rationalized on the basis of nervous components which respond to a lowering of temperature by an increase in electrical activity. Cold receptors are well documented in mammals, and functionally similar sense receptors probably occur in fish. The pathways through which this increased electrical activity could result in increased locomotor activity are not difficult to visualize.

Whether or not these overshoot responses of fish to temperature change have an ecological significance is an open question.

ACTIVITY IN THE STABILIZED PHASE

The slope of the activity vs. temperature curves (Fig. 9) for fish in the stabilized phase in general resemble those obtained for brook trout (Fisher and Sullivan, 1958). They differ only in that for trout two maxima were obtained, the second maximum occurring just before the upper lethal temperature was reached. For the salmon acclimated to 18 C the activity was still progressing upwards at 27 C, although this temperature would undoubtedly be lethal within 24 hr or so. Fisher and Sullivan (1958) also observed that the first maximum corresponded closely to the temperature selected in a horizontal gradient by their fish. The selected temperature of salmon acclimated to 6 C is about 8 C, and for salmon acclimated to 18 C is about 17 C (Javaid and Anderson, 1967). These temperatures are not the ones at which the activity maxima occur (Fig. 9). Perhaps if the test temperatures had been spaced closer together in the vicinity of the plateau regions, the temperatures corresponding to peaks in activity would have more closely approximated the selected temperature values. About all we can say is that the activity peaks occur at temperatures which are near the selected temperatures. Also, increasing the acclimation temperature appears to shift the temperature at which the activity maxima occur rightwards (i.e. warmwards), the same direction in which the selected temperature is shifted by increasing acclimation temperature in Atlantic salmon.

LONG-TERM RESPONSES TO TEMPERATURE CHANGE

The abrupt increase in weight for fish transferred to 6 from 18 C (Fig. 10) cannot be due to growth or increase in gut contents since the fish refused to feed for the 1st day or two after the temperature change. In all probability, it is due to water uptake, since such a response to a rapid temperature drop has been reported (Hickman et al., 1964). This weight gain is lost after 4–6 days. Subsequent increase in weight is presumably due to growth.

In view of the small number of fish tested, the initial fluctuations in standard oxygen consumption of the fish transferred from 18 to 6 C (Fig. 11) may not be valid. Beamish (1964) has shown that the standard oxygen consumption falls during starvation, so possibly the low values on the 4th and 6th days are due to starvation. The fish ate little during the 1st day or two, and lost weight up to the 4th day. The weight increased steadily after 4 days. No initial abrupt increase in weight was observed, as was noted when salmon acclimated to 18 C were placed into 6 C. Since 48 hr elapsed before the first measurement was taken, it is possible that a weight increase did occur, but was missed. Goldfish have been found to absorb water upon rapid temperature increase (Heinicke and Houston, 1965); however, the water content built up slowly over 10 days.

The results for the time-course of acclimating (Fig. 10 and 11) suggest that the time required for full acclimation of standard oxygen consumption, and probably spontaneous activity as well, under the stated conditions, is about 2 weeks or less. There is no evidence to indicate that there is any difference in the time required for acclimation to a colder than to a warmer temperature.

The fact that the acclimation rates observed in these experiments are independent of the direction of the temperature change warrants attention. It is well established for fish that heat tolerance is gained rapidly after a rise in acclimation temperature but is lost slowly after cooling (Doudoroff, 1942; Brett, 1944, 1946). Cold tolerance appears to be acquired no more slowly than it is lost, although in comparison with the work done on heat tolerance, the times are relatively long, e.g., more than 20 days for *Girella nigricans* subjected to a change in acclimation temperature from 26 to 14 C (Doudoroff, 1942). If one hypothesizes that a biochemical reaction(s) underlies thermal acclimation processes, then the results are reasonable. Thermo-chemical reactions have a Q_{10} between 2 and 5 and one would expect this fact to be reflected in longer times being required for the completion of any process occurring at lower temperatures. However, when one measures the rate of chemical reactions directly, as we have done here with oxygen consumption, the generalization emerges, consistent with our results, that although there may be considerable variations in the rates depending on the system and species studied, metabolic thermal acclimation takes no longer for a warm-to-cold than for a cold-to-warm change in acclimation temperature (Schultze, 1965; Baslow and Nigrelli, 1964). Klicka (1965) reported that thermal acclimation of oxygen consumption of goldfish took *longer* for a change from 15 to 30 C than for the reverse change (12 days vs. 3 days), but since he did not indicate the fraction of his oxygen consumption values which was related to spontaneous activity, his results are at best equivocal in the present context. Temperature selection is another process showing a thermal acclimation response in which the rate of change of the acclimation response appears to be independent of the direction of the temperature change (Doudoroff, 1938; Javaid, MS, 1967).

If changes in such physical processes as diffusion or conductivity are responsible for thermal acclimation processes, then time differences in acclimation to high and low temperatures might not be easily observed since the Q_{10} would be only about 1.3. Perhaps, however, the most reasonable explanation for the difficulty in interpreting the present data is that there is no a priori reason why acclimation to high temperature has to be the exact reverse process of acclimation to low temperature.

ACKNOWLEDGMENTS

It is a pleasure to acknowledge the generous support of the Saint John, N.B., hatchery of the Canada Department of Fisheries for providing the fish used in these experiments. R.H.P. was assisted by scholarships from the National Research Council of Canada. J.M.A. was given valuable grants-in-aid from the Fisheries Research Board of Canada and the National Research Council of Canada.

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Folic Acid Anemia in Coho Salmon

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Western Fish Nutrition Laboratory
U.S. Fish and Wildlife Service, Cook, Washington

Smith, Charlie E., and John E. Halver. 1969. *Folic acid anemia in coho salmon.* J. Fish. Res. Bd. Canada 26: 111–114.

Juvenile coho salmon (*Oncorhynchus kisutch*) fed a folic acid-deficient diet and sampled at 6, 9, 12, and 14 weeks developed macrocytic anemia. The anemia, first observed at 6 weeks, was characterized by a significant reduction in red blood cell count as well as macrocytosis and poikilocytosis of erythrocytes. The abnormally shaped erythrocytes observed in peripheral blood smears may be an important aid in the identification of folic acid deficiency in coho salmon. Gross manifestations of the deficiency were extremely pale gills; exophthalmia, often accompanied by ascites fluid; dark coloration; and reduction in growth. Fish recovered after 8 weeks on a diet adequate in folic acid and exhibited a normal blood picture.

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INTRODUCTION

Smith (1968) reported that coho salmon (*Oncorhynchus kisutch*) developed megaloblastic, normochromic macrocytic anemia after 24 weeks if fed a diet in which the only source of folic acid was that present in the natural ingredients of a commercially prepared diet. The anemia was further characterized by anisocytosis and poikilocytosis of erythrocytes. This report substantiates the earlier findings and describes the periodic changes in the blood picture of coho salmon fed a water soluble vitamin test diet from which folic acid was omitted.

METHODS

The experiment was conducted on coho salmon at the Western Fish Nutrition Laboratory, Cook, Washington, where well water temperature is maintained constant at 10 C. Control and test fish (average weight 0.70 g) were distributed into duplicate troughs of 300 fish each and were fed twice daily, 6 days weekly. Control fish received the complete test diet (CTD) (Halver, 1957); test fish received the same diet with folic acid omitted. Each group was weighed biweekly, and troughs were thoroughly scrubbed during the weighing period to remove any accumulated algae.

Blood samples were removed from 10 deficient and 10 control fish at the end of 6, 9, and 12 weeks. After 14 weeks, 50 samples were taken from each group. At this time, in order to determine rate of recovery, the remaining population was divided into three lots and treated in the following manner: lot 1 was injected with 20 μg folic acid and then fed CTD; lot 2 was injected with saline and then fed CTD; and lot 3 continued on folic acid-deficient diet. Both recovery groups (lots 1 and 2) were sampled after 4 and 8 weeks. Those that continued on the folic acid-deficient diet were sampled only after the 4-week recovery period or after 18 weeks on the deficient diet.

Red blood cell (RBC) counts were determined by the method of Hesser (1960). Blood smears and imprints of hemopoietic kidney were prepared and stained as reported previously (Smith,

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1968). Differences in RBC size were determined with an ocular micrometer by measuring 10 normal-appearing, mature erythrocytes from stained blood smears prepared from each of five fish at time of sampling. The Student's *t*-test was used to determine the significance of differences.

RESULTS

Manifestations of the deficiency syndrome were first observed in the deficient population at the 6-week sampling period. Gills and blood were both pale when compared with those of control fish. These symptoms increased in severity until the experiment was terminated (Fig. 1).

Reduction in growth rate was noted by the end of 8 weeks on test. By the end of 12 weeks, mortality had begun to occur and several darkly pigmented fish were noted. After 14 weeks, deficient fish ate very little, mortality was increased, and darkly pigmented fish were common. Several fish exhibited exophthalmia alone or in combination with abdomens distended with ascites fluid.

Average RBC counts and cell sizes of control and deficient salmon at time of sampling are listed in Table 1. Both values showed a significant difference at 6 weeks when compared with those of the controls. However, whereas the anemia increased in severity with time, RBC size remained quite constant after the first sampling.

TABLE 1. Effects of folic acid deficiency on average red blood cell (RBC) counts and RBC length of juvenile coho salmon.

No. weeks on diet	RBC's/mm^3 blood		RBC length (μ)	
	Controls	Deficient	Controls	Deficient
6	860,000	498,000[b]	14.8	16.5[b]
	(680,000–1,120,000)[a]	(310,000–650,000)	(13–16)[a]	(12–22)
9	1,200,000	384,000[b]	15.3	16.5[c]
	(950,000–1,380,000)	(200,000–530,000)	(13–17)	(14–20)
12	950,000	190,000[b]	15.1	16.9[b]
	(800,000–1,260,000)	(120,000–270,000)	(13–17)	(15–19)
14	990,000	146,000[b]	15.1	16.9[d]
	(790,000–1,210,000)	(40,000–280,000)	(14–16)	(15–20)

[a] Range.
[b] $P < .01$ control vs. deficient.
[c] $P < .02$ control vs. deficient.
[d] $P < .05$ control vs. deficient.

Examination of blood smears prepared at 6 weeks revealed anisocytosis and poikilocytosis as well as segmentation and constriction of RBC nuclei. The abnormal cells observed at this sample period were usually immature (Fig. 2–6). As the anemia increased in severity, at 9, 12, and 14 weeks the

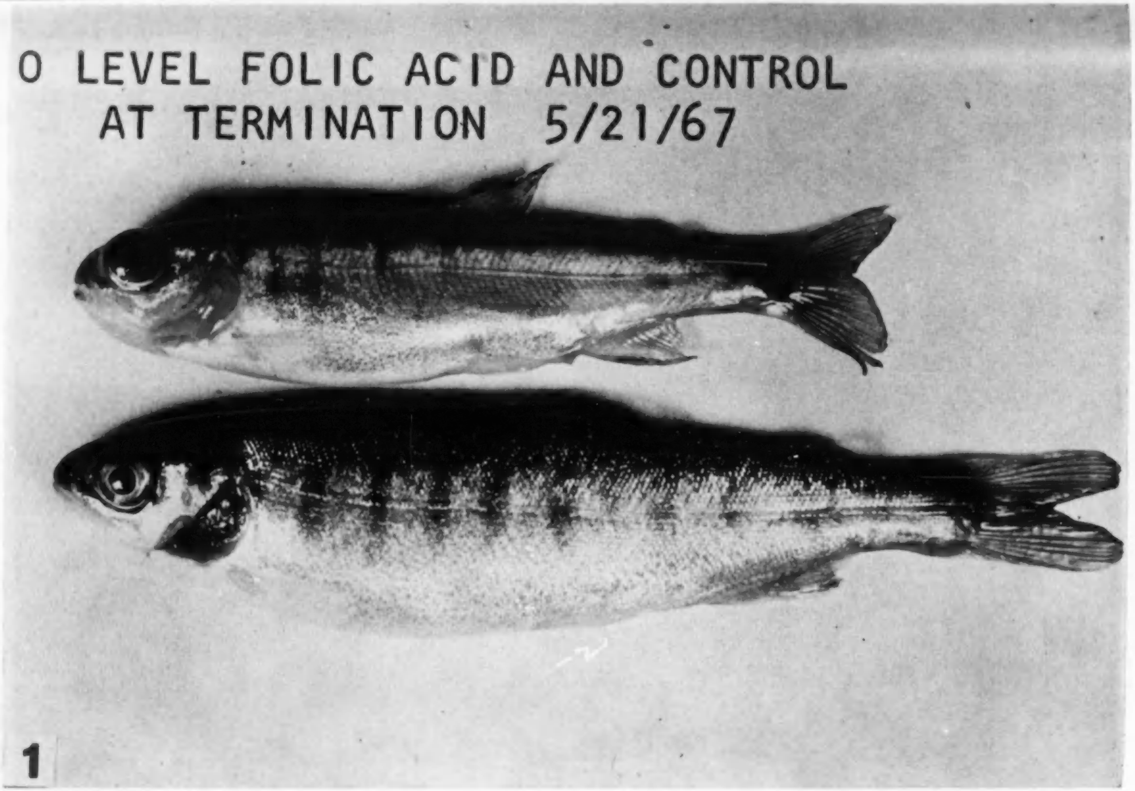
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FIG. 1. Control and folic acid-deficient coho salmon, 12 weeks. Note size difference, pale gills, exophthalmia, and coloration of deficient.

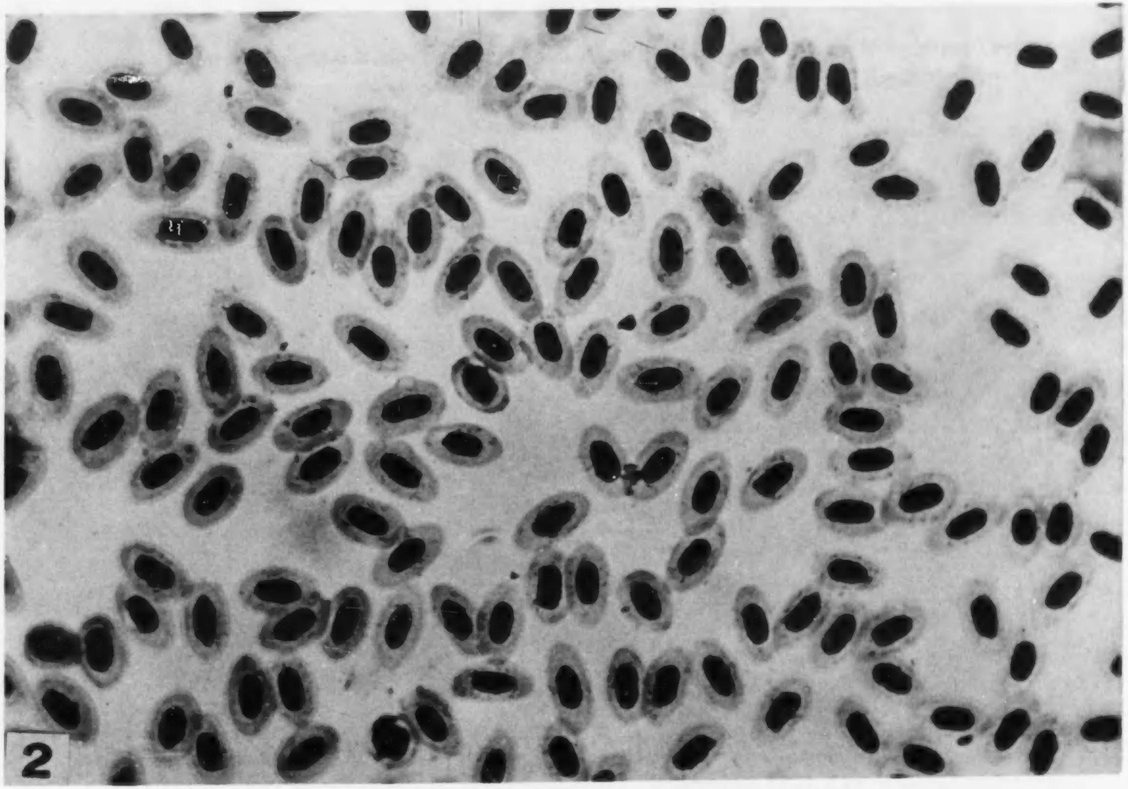
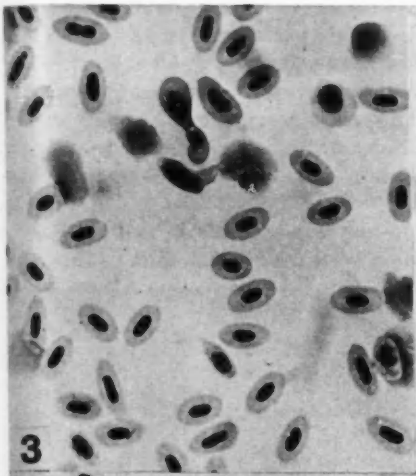
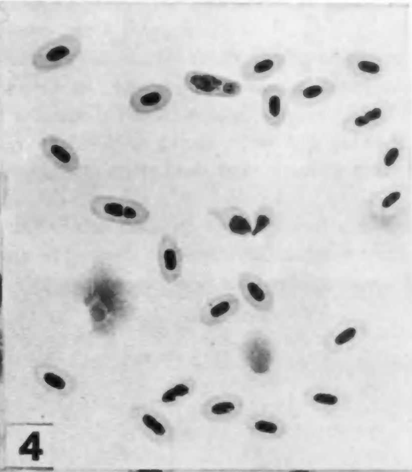
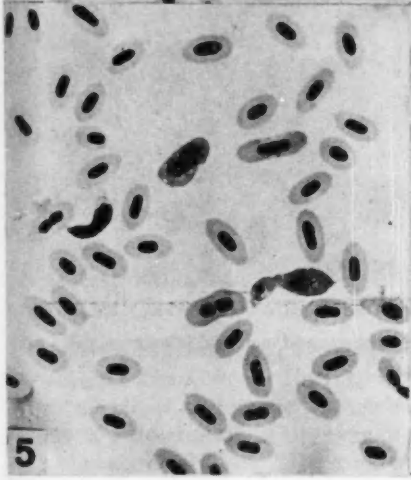


FIG. 2. Normal RBC's from control coho salmon. ×1000.







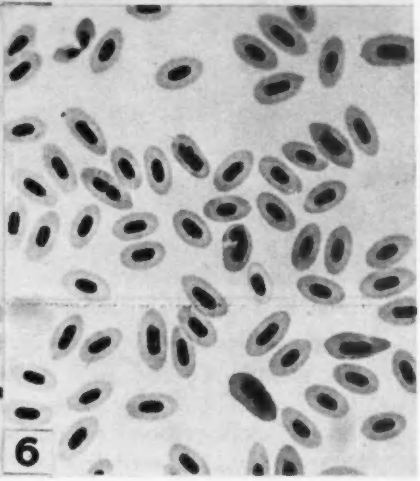
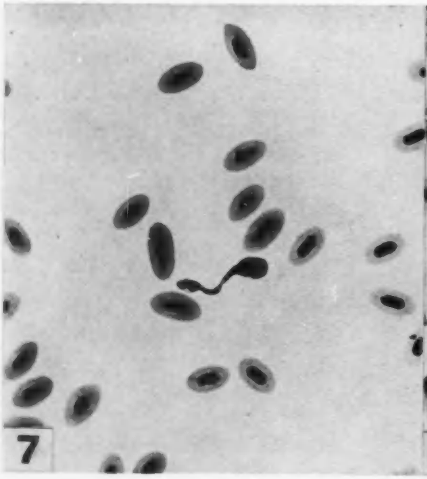


FIG. 3. Anisocytosis and poikilocytosis of immature RBC's. Folic acid-deficient coho, 6 weeks. ×325.

FIG. 4. Anisocytosis and poikilocytosis of immature RBC's. Note segmentation of some nuclei. Folic acid-deficient coho, 6 weeks. ×325.

FIG. 5. Same as Fig. 4.

FIG. 6. Anisocytosis and nuclear segmentation of RBC's. Folic acid-deficient coho, 6 weeks. ×325.



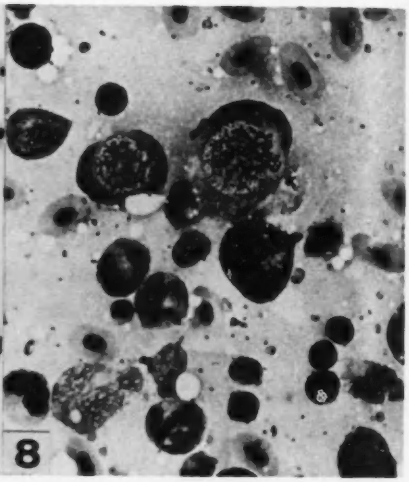


FIG. 7. Poikilocytic erythrocyte. Folic acid-deficient coho, 9 weeks. ×325.

FIG. 8. Megaloblastic erythroid precursors Note large size and increased interchromatin spaces. Folic acid-deficient coho, 6 weeks. ×800.

defect was more common in mature erythrocytes (Fig. 7). Red blood cell production appeared to decrease as time on the deficient diet increased since few immature cells were present in blood smears after 9 weeks.

A similar finding was observed in kidney imprints. At 6 weeks numerous erythroid precursor cells were present. Some of these cells were megaloblastic in that there was an increase in size and nuclear interchromatin spaces (Fig. 8). As time on the deficient diet increased, erythrocyte production was retarded and fewer erythroid precursor cells were present, and these were usually megaloblastic.

The effect of the recovery diet on RBC counts and average cell size can be seen in Table 2. Although RBC counts appeared to be normal after 4 weeks on the diet, 8 weeks lapsed before blood cell morphology had returned to normal.

TABLE 2. Effects of recovery diet on average red blood cell (RBC) counts and RBC length of juvenile coho salmon.

Avg RBC count			
18 weeks on folic acid-deficient diet	4 weeks on recovery diet	8 weeks on recovery diet	RBC length after 8 weeks on recovery diet (μ)
180,000	1,140,000[a]	1,420,000[a]	15.4
	1,030,000[b]	1,500,000[b]	(13–17)[c]

[a]Injected with 20 μg folic acid.
[b]Injected with saline.
[c]Range.

DISCUSSION

Previous investigators describing the folic acid requirements of chinook salmon and brook trout (Halver, 1957; Phillips, 1963; Phillips et al., 1961) described slight and moderate anemia respectively in folic acid deficiency. However, a description of the type of anemia was not reported.

The extremely low RBC counts obtained from fish in this experiment suggests that blood cell formation in coho salmon is very sensitive to folic acid deficiency. Neither chinook salmon nor brook trout exhibited such a depression in RBC counts.

The abnormally shaped erythrocytes and nuclear abberations noted in blood smears apparently resulted from the lack of folic acid which is involved in the incorporation of nucleotides into DNA. Deficiency may lead to impaired replication of DNA, thus leading to the morphological changes that were observed. It seems apparent that detection of these abnormally shaped cells in combination with changes in other hematological parameters can serve as important clinical tools in the diagnosis of folic acid deficiency in coho salmon.

CONCLUSIONS

Coho salmon developed megaloblastic, macrocytic anemia when fed a diet deficient in folic acid. This confirms the finding of a previous experiment and is typical of the anemia that develops in several other animals with folic acid deficiency.

ACKNOWLEDGMENTS

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Some Metabolic Effects of Bacterial Endotoxins in Salmonid Fishes

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Coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) were highly resistant to endotoxins from both *Escherichia coli* and *Aeromonas salmonicida* (a fish pathogen) at 14 and 18 C.

This resistance was investigated with liver tryptophan pyrrolase, liver glycogen depletion in vitro, and the arterial blood pressure as indicators. Liver glycogen depletion was accelerated by both endotoxins, but there was no significant cardiovascular response or effect on liver tryptophan pyrrolase activity. Since the cardiovascular effects of histamine were also limited, it was concluded that the metabolic effects of bacterial endotoxins in salmonids are qualitatively different from those of the higher vertebrates.

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INTRODUCTION

THE LETHAL EFFECTS of the gram-negative bacterial diseases of mammals are commonly considered to be caused by lipopolysaccharide endotoxins released from the cell walls of lysed microorganisms. Some of the endotoxin-induced metabolic derangements which have been implicated in the subsequent death of the host include: depletion of liver glycogen, depression of liver tryptophan pyrrolase activity, and severe hypotension followed by shock, presumably caused by the endotoxin-mediated release of histamine from mast cells (Zweifach and Janoff, 1965).

Though most of the bacterial diseases of fishes are due to gram-negative microorganisms, the physiologic effects of the endotoxins of these microorganisms have not been studied as such. Berczi et al. (1966) reported *Escherichia coli* endotoxin to be less toxic to fishes than to amphibians and the higher vertebrates, but liver glycogen, tryptophan pyrrolase function, or other metabolic activities were not measured. Significantly, amphibians as well as certain

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endotoxin-resistant higher vertebrates are also highly refractory toward the effects of circulating histamine and other compounds whose effects are mediated by β-adrenergic receptors (Reite, 1965; Zweifach and Janoff, 1965). Unfortunately, nothing is known of the vascular effects of histamine in salmonids, though it is known that vascular β-adrenergic receptors are absent (Randall and Stevens, 1967).

This investigation was designed to determine the response of salmonids to bacterial endotoxins as indicated by liver glycolytic activity, tryptophan pyrrolase activity, and the effect on arterial blood pressure. The endotoxins chosen for this study were lipopolysaccharide B, *E. coli* 055-B5 (Difco) commonly used as a standard, and an endotoxin extracted by one of us (A.J.R.) from *Aeromonas salmonicida*, a fish pathogen. Coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) were used as test organisms since *A. salmonicida* is pathogenic for both (furunculosis disease).

METHODS AND MATERIALS

GLYCOGEN DEPLETION IN VITRO

Livers, from 1- to 2-year-old coho salmon or rainbow trout held in fresh water at 14 C, were pooled and homogenized for 2 min with seven volumes of cold 0.07 M phosphate buffer, *p*H 7.0, 0.14 M in KCl. One ml of the homogenate was added to 1 ml of 0.07 M phosphate buffer, *p*H 7.0, containing the desired amount of endotoxin (usually 0.5 mg) and the mixture incubated with shaking at 25 C. Endotoxin was omitted in the control mixtures. At 0, 2, 4, and 6 hr, aliquots were withdrawn, boiled with 3 ml of 30% KOH to dissolve the tissue, and analyzed for glycogen by the anthrone method (Roe and Dailey, 1966).

TRYPTOPHAN PYRROLASE

Coho salmon, held at 14 C, were injected with 0.1 ml of saline containing the desired amount of endotoxin. The saline was injected either into the branchial artery by the technique of Ames (1966), or subcutaneously anterior to the dorsal fin. At stated times post-treatment, liver tryptophan pyrrolase activity was determined by the method of Knox (1955), with an incubation temperature of 25 C.

BLOOD PRESSURE

Rainbow trout weighing 400–500 g were anesthetized with 100 ppm tricaine methanesulfonate (M.S. 222) and their dorsal aortas were cannulated by the technique of Smith and Bell (1964). After the fish recovered from the anesthetic sufficiently to swim normally, enough endotoxin or histamine, in 1 ml of physiological saline, was flushed through the cannula to yield a final dosage of 5 or 100 mg/kg, respectively. Arterial blood pressure changes were followed with a Clevite-Brush oscillograph connected to the cannula through a pressure transducer. Control fish were injected in the same manner with up to 4 ml of saline to determine the effect of the injection itself on the arterial pressure.

PREPARATION OF ENDOTOXIN

Endotoxin was extracted from log-phase *A. salmonicida* cells by the hot phenol–water method of Westphal et al. (1952) except that additional purification steps were omitted. The resulting material was assayed for activity, by means of the chick embryo and rabbit pyrogenicity test, by the Rocky Mountain Laboratory of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. Their results indicated a highly potent soluble endotoxin. The *E. coli* endotoxin used was purchased commercially (Difco), and was not assayed for activity prior to use.

RESULTS AND DISCUSSION

The extreme resistance to *E. coli* endotoxin exhibited by carp (Berczi et al., 1966) was also true for yearling coho salmon and rainbow trout with both the *A. salmonicida* and *E. coli* endotoxins. Intravascular or subcutaneous injections of up to 80 mg/kg of either endotoxin had no visible effect on these fish at water temperatures of 14 or 18 C. Thus, an LD_{50} could not be established.

To clarify these results, liver tryptophan pyrrolase activity in coho salmon injected subcutaneously with 0.1–40 mg/kg of *E. coli* endotoxin was determined. However, the levels found were not substantially different from the saline-injected controls when assayed 24 hr post-injection (Table 1). In addition, when tryptophan pyrrolase activity was measured as a function of time no significant changes in level occurred over a period of 65 hr post-injection using a 10-mg/kg dosage (Table 2). Significantly, dexamethasone (cortisone) injections singly or in combination with endotoxin had no effect on liver tryptophan pyrrolase activity (Table 3). These results may partially explain the extreme endotoxin resistance displayed by these fish. By comparison, in the rat the level of this key enzyme and thus the level of di- and triphosphopyridine nucleotides is elevated by cortisone and strongly depressed by even small amounts of several endotoxins (Berry and Smythe, 1963).

TABLE 1. Tryptophan pyrrolase activity vs. amount of *Escherichia coli* endotoxin injected subcutaneously into coho salmon at 14 C; livers analysed 24 hr post-injection. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Injected (*mg/kg*)	Trytophan pyrrolase activity (μM kynurenine/hr per g tissue)
0 (controls)	8.9 ± 2.1(15)
0.1	5.6 ± 0.7(12)
2	10.8 ± 4.2(10)
10	7.2 ± 1.1(18)
40	6.0 ± 3.3(16)

Endotoxin-induced liver glycogen depletion has been widely studied in mammals. It is generally considered to be due to glycogen phosphorylase activation, and has been the only endotoxic effect found to occur in vitro. Our data (Fig. 1, 2, 3) confirm that this effect also operates in the coho salmon. A moderate but reproducible dose-dependent acceleration of glycogenolysis in vitro occurred with both *E. coli* and *A. salmonicida* endotoxins. Figure 1 demonstrates accelerated glycogenolysis by *A. salmonicida*, and Fig. 2 and 3 show dose effects of the two toxins. Results obtained with *A. salmonicida* endotoxin (Fig. 3) were somewhat unusual in that a progressive inhibition of glycogenolysis occurred as the endotoxin concentration increased, a phenomenon presumably due to impurities since the *E. coli* endotoxin preparation did not show this effect. In vivo glycogen depletion tests were carried out but were

TABLE 2. Tryptophan pyrrolase activity as a function of time after subcutaneous *Escherichia coli* endotoxin injection into coho salmon at 14 C, 10 mg/kg dosage. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Time post-injection (*hr*)	Tryptophan pyrrolase activity (μM kynurenine/hr per g tissue)
0 (controls)	9.7±3.5(10)
5	16.5±3.6(12)
11	13.6±2.9(11)
24	11.9±2.7(12)
48	15.7±3.3(12)
65	14.8±2.4(16)
65 (controls)	10.6±1.7(16)

TABLE 3. Effect of subcutaneous *Escherichia coli* endotoxin and cortisone injections singly and in combination on liver tryptophan pyrrolase activity in coho salmon; 14 C, livers analyzed 24 hr post-injection. Each value is the mean ± standard deviation for the number of determinations shown in parentheses.

Exptl. treatment	Tryptophan pyrrolase activity (μM kynurenine/hr per g tissue)
Controls	10.7±3.5(32)
Cortisone only (5 mg/kg)	11.1±2.9(12)
Endotoxin only (10 mg/kg)	8.2±2.1(12)
Endotoxin + cortisone	8.2±3.1(12)

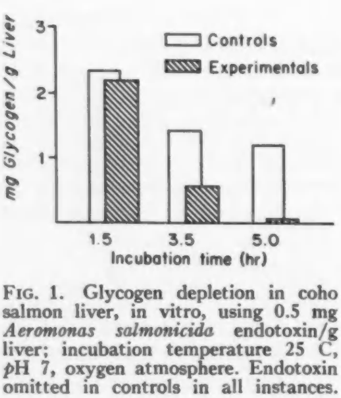

FIG. 1. Glycogen depletion in coho salmon liver, in vitro, using 0.5 mg *Aeromonas salmonicida* endotoxin/g liver; incubation temperature 25 C, *p*H 7, oxygen atmosphere. Endotoxin omitted in controls in all instances.

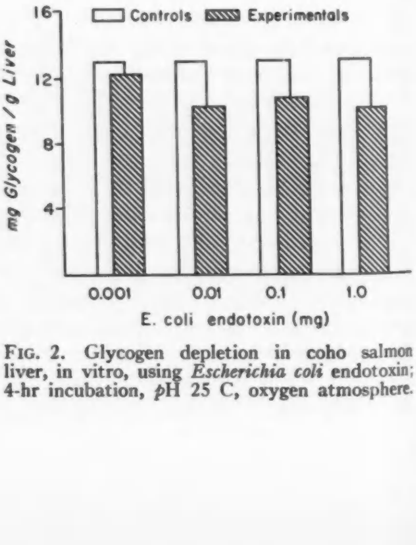

FIG. 2. Glycogen depletion in coho salmon liver, in vitro, using *Escherichia coli* endotoxin; 4-hr incubation, *p*H 25 C, oxygen atmosphere.

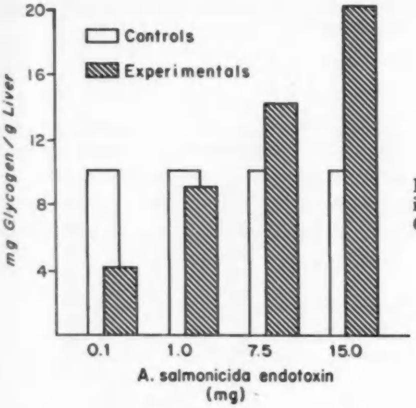

FIG 3. Glycogen depletion in coho salmon liver, in vitro, using *Aeromonas salmonicida* endotoxin; 6-hr incubation, pH 7, 25 C, oxygen atmosphere.

inconclusive because of the wide variation in normal liver glycogen levels between individual fish. Thus, although Woods et al. (1961) have postulated that the biochemical pathology of endotoxins centers around their glycolytic activity, the lack of an acute endotoxemia in these fish tends to belie the importance of this in vitro effect. In addition, the consensus is that glycolytic activity is not of central importance in accounting for endotoxin toxicities (Zweifach and Janoff, 1965).

In the higher vertebrates, the hypotensive effect of endotoxins is immediate, a precipitous decline in arterial pressure normally occurring within a few seconds of endotoxin administration (Brockman et al., 1967). However, as the data in Tables 4 and 5 show, both the *E. coli* and the *A. salmonicida* endotoxins were virtually without effect on the arterial blood pressure of rainbow trout over a 30-min period. In addition, the abrupt reduction in heart rate commonly seen in mammals (Levy and Blattberg, 1967) did not occur.

TABLE 4. Hemodynamic responses of rainbow trout given *Escherichia coli* endotoxin. Water temperature 18 C. Intravascular endotoxin injection at zero time.

	5 mg/kg		10 mg/kg	
Elapsed time (*min*)	Heart rate (*beats/min*)	Systolic pressure (*mm Hg*)	Heart rate (*beats/min*)	Systolic pressure (*mm Hg*)
–2	84	20	96	19
–1	70	18	90	19
0	84	20	96	17
1	84	20	108	17
2	84	20	96	16
5	84	18	123	16
10	56	18	99	17
20	44	18	105	17
30	20	15	105	17

Table 5. Hemodynamic response of rainbow trout given *Aeromonas salmonicida* endotoxin. Water temperature 18 C. Intravascular endotoxin injection at zero time.

	20 mg/kg		47 mg/kg	
Elapsed time (*min*)	Heart rate (*beats/min*)	Systolic pressure (*mm Hg*)	Heart rate (*beats/min*)	Systolic pressure (*mm Hg*)
−2	84	25	87	24
−1	84	28	87	24
0	60	28	126	24
1	84	32	84	20
2	84	28	84	20
5	96	28	84	20
10	84	28	84	24
20	84	28	84	24
30	84	28	84	23

The blood pressure data obtained would also presumably apply to coho salmon since *A. salmonicida* is pathogenic for both genera; unfortunately, only rainbow trout could be used because hatchery-reared coho salmon are not large enough to cannulate reliably.

As previously mentioned, histamine (Hm) released from mast cells by bacterial endotoxins is thought to mediate the arterial hypotension and shock which characteristically develop during severe gram-negative bacterial infections of the higher vertebrates. Since fishes are evidently refractory toward injected endotoxins, their cardiovascular response to Hm is potentially important in accounting for both this resistance and the lethality of their gram-negative bacterial diseases.

Unfortunately, the effects of Hm on the vascular system of fishes have not been well documented. Early workers generalized that a cardiovascular response to Hm is either absent or quite unimportant in poikilothermic vertebrates (Feldberg and Schilf, 1930). More recently Reite (1965) found tissue Hm levels of less than 1 μg/g in both primitive and evolutionally advanced fishes, and concluded that endogenous Hm was probably unimportant in their metabolism even though mast cells are normally present. However, Mott (1951) has reported that only 2 μg of Hm will cause marked hypotension in the eel (*Anguilla anguilla*), one of the few vertebrates completely lacking in mast cells (Reite, 1965).

Preliminary experiments using 15 coho salmon and rainbow trout weighing 50–200 g revealed that Hm injected subcutaneously or into the branchial artery caused immediate opercular flaring (gasping), loss of equilibrium, and thrashing, in a dose-dependent manner. Since the threshold for these grossly visible effects was about 100 mg/kg, with death occurring only after very large (3000 mg/kg) dosages, it appeared unlikely that histamine could be important in an endotoxemia. However, to examine the response in more detail, arterial blood pressure measurements were made on two 500-g rainbow trout by the previously described method. After the fish were swimming normally and a base line was established, enough Hm in 1 ml of physiological saline was flushed through the cannula to yield a dosage of 100 mg/kg. Previous experiments had shown that under these conditions up to 4 ml of saline could be injected into the dorsal aorta of these fish with no measurable effect on blood pressure.

Characteristically, there was a rapid reduction in both systolic and diastolic pressure followed by a slow increase, over 30 min, to approximately the initial level (Table 6). As expected, the heart rate increased significantly during the initial period of hypotension and then became quite irregular. However, the pulse pressure (systolic–diastolic) did not vary appreciably. There were many temporary fluctuations in systolic and diastolic pressure, caused by random thrashing, but these did not grossly affect the general trend (Fig. 4). The arterial pressure could be raised, momentarily, by 4 mg/kg of the antihistaminic diphenhydramine hydrochloride (Benadryl) given in 0.2 ml of saline. This volume of saline alone had no effect on the arterial pressure.

TABLE 6. Hemodynamic response of rainbow trout to 100 mg histamine/kg. Water temperature 18 C. Intravascular injection of histamine at zero time.

Elapsed time	Heart rate (*beats/min*)	Systolic pressure (*mm Hg*)
−10 sec	96	32
0 "	132	8
+10 "	132	8
+30 "	24	12
+ 2 min	Irregular (72–96)	15
+ 5 "	48	15
+10 "	60	18
+28 "	84	26

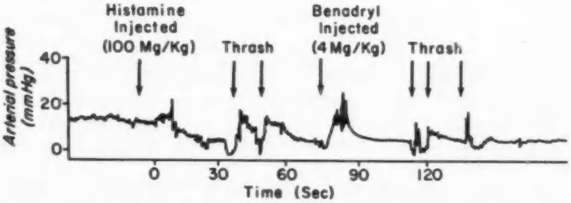

FIG. 4. Early blood pressure changes in the rainbow trout following histamine injection. Water temperature 18 C. The antihistaminic Benadryl temporarily normalized the arterial pressure, but the large dose required was toxic.

Thus, it is evident that rainbow trout, and probably other salmonids as well, do respond to circulating Hm, and in a manner grossly analogous to the higher vertebrates. However, Hm involvement in the endotoxic response (and hence in the gram-negative bacterial diseases) of salmonids must probably be ruled out because of the excessively large and hence nonphysiological dosages required to elicit significant arterial hypotension.

In conclusion, the resistance of salmonid fishes to purified endotoxins can be at least partially accounted for by the type of histamine response which occurs (presumably due to the absence of β-adrenergic receptors) and by the lack of any liver tryptophan pyrrolase response. Although liver glycogen depletion did occur in vitro and Woods et al. (1961) have postulated that the lethal effects of endotoxins center around glycogenolysis, the consensus is that this effect is probably not of primary importance (Zweifach and Janoff, 1965).

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Allometric Weight–Length Regression Model

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Pienaar, L. V., and J. A. Thomson. 1969. *Allometric weight–length regression model.* J. Fish. Res. Bd. Canada 26: 123–131.

Two methods of fitting the allometric weight–length relationship are described, one involving the common logarithmic transformation of variables in a multiplicative model and the other assuming an additive nonlinear model and general nonlinear estimation procedures. Differences in the assumptions involved in the two methods are emphasized and the practical significance of the different methods is demonstrated with the aid of a sample problem. A number of procedures are suggested to compensate for possibly unjustified assumptions.

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INTRODUCTION

Commonly, in the study of the dynamics of a fish population, one of the initial steps is the derivation of a relationship between the weight and length of a fish. It is recognized that the nature of this relationship is statistical in the sense that there is no exact mathematical relationship between weight and length and that the inherent variation in weight between individuals of the same length is large compared with the errors of measurement. It is also known that, on the average, weight tends to increase with length and we therefore expect to be able to predict the average weight of individuals of a given length.

The relationship between weight and length of fish is commonly derived by means of regression analysis, which requires a specified model of what is believed to be the mathematical form of the relationship. In some cases the major consideration in the derivation of a regression model is the particular configuration of the data at hand, as exhibited in some appropriate plot; in other cases the formulation of the model is not prescribed in such a direct manner by the peculiarities of a sample, but rather in terms of a logical hypothesis concerning the nature of the relationship. In the case of the weight–length relationship it has been observed, for example, that for many fish species a linear model was inappropriate over the range of lengths normally encountered, because the increase in weight over short periods of time is not proportional to the increase in length. It seems more appropriate to assert that the relative rate of growth in weight is proportional to the relative rate of growth in length. In terms of instantaneous rates the assumed model is

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$$\frac{d(ln\,\mathrm{W})}{dt} = \beta . \frac{d(ln\,\mathrm{L})}{dt}$$

which integrates to

$$\mathrm{W} = \alpha \mathrm{L}^{\beta} \qquad (1)$$

where W is weight, L is length, and α and β are unknown parameters.

Relationships of the general form of equation 1 are commonly referred to as allometric relationships. Paulik and Gales (1964) have also derived this form of the weight–length relationship for the general case of allometric growth in linear body dimensions. The model of equation 1 is accepted as reasonable and has been demonstrated in practice to be of sufficient flexibility to provide an acceptable fit to a great variety of sample data. It will be regarded here as an appropriate formulation of the true underlying relationship between weight and length, and that its parameters α and β are characteristics of a particular population.

Suppose that a random sample of individuals has been weighed and measured and the objective is the prediction of average weights from given lengths, for which we require estimates of the unknown parameters α and β. We shall demonstrate how different estimation procedures, involving different assumptions, affect the final results, and comment on the justifiability of various assumptions. The weights and lengths of a random sample of tagged and recovered Pacific cod (*Gadus macrocephalus*), given in Table 1, were used for this purpose. There were 43 (n) random pairs of observations (W_i, L_i), i = 1, 2,. . . . ., n.

METHOD 1

Perhaps the most common procedure that involves a regression model of the form of equation 1 is to regard this as a multiplicative model that is intrinsically linear, i.e.

$$\mathrm{W}_i = \alpha \mathrm{L}_i^{\beta}\, \epsilon_i$$

so that

$$\log \mathrm{W}_i = \log \alpha + \beta \log \mathrm{L}_i + \log \epsilon_i \qquad (2)$$

or

$$\mathrm{Y}_i = \alpha' + \beta\, \mathrm{X}_i + \delta_i$$

in an obvious notation. The model as expressed in equation 2 is linear in the parameters α' and β and standard linear regression methods can be applied to the transformed variables. It is assumed that ϵ_i is a multiplicative random error from a log normal distribution with zero mean, i.e. δ_i is $\mathrm{N}(0, \sigma^2)$. In matrix notation 2 is written as

$$\mathbf{Y} + \mathbf{X}\, \boldsymbol{\theta} + \boldsymbol{\delta} \qquad (3)$$

where $\theta = [\alpha', \beta]'$. The least squares estimator of $\boldsymbol{\theta}$ is given by

$$\hat{\boldsymbol{\theta}} = (\mathbf{X}'\mathbf{X})^{-1}\, \mathbf{X}'\, \mathbf{Y} \qquad (4).$$

If it is assumed that

$$\begin{aligned} E(\boldsymbol{\delta}) &= \mathbf{0} \\ \text{and} \quad E(\boldsymbol{\delta}\boldsymbol{\delta}') &= \sigma^2 \, \mathbf{I} \end{aligned}$$

then the least squares estimator of equation 4 is the best linear unbiased estimator. It is emphasized that the necessary assumptions are that $E(\boldsymbol{\delta}) = \mathbf{0}$ and that $E[\boldsymbol{\delta}\boldsymbol{\delta}'] = \sigma^2 \, \mathbf{I}$, rather than $\boldsymbol{\epsilon}$.

TABLE 1. Length–weight data.

Length (*cm*)	Weight (*oz*)
45.2	24.0
45.7	57.6
47.2	27.2
48.8	76.8
50.5	44.8
51.2	70.4
53.0	91.2
53.7	38.4
54.0	73.6
56.0	96.0
56.1	110.4
56.1	49.6
57.5	73.6
58.0	49.6
59.4	96.0
59.8	67.2
60.8	81.6
61.0	118.4
61.5	131.2
61.9	64.0
62.9	124.8
63.0	80.0
65.0	129.6
65.0	78.4
66.0	155.2
66.2	113.6
66.6	100.8
68.0	139.2
69.3	158.4
69.4	131.2
70.2	116.8
70.6	153.6
71.0	99.2
71.9	177.6
72.6	126.4
73.8	184.0
73.8	201.6
74.0	164.8
75.7	142.4
75.8	192.0
76.2	161.6
76.6	204.8
77.3	139.2

The least squares estimator obtained from equation 4, using the data of Table 1, was

$$\hat{\boldsymbol{\theta}}' = [-3\cdot 23027, \; 2\cdot 90660]$$

so that

$$\log \hat{W}_i = -3\cdot 23027 + 2\cdot 90660 \log L_i$$

or

$$\hat{W}_i = 0\cdot 00058847 \; L_i^{2\cdot 90660} \qquad (5).$$

The data points of Table 1 and the fitted curve of equation 5 are shown in Fig. 1 (broken line). Some appreciation of the validity of the assumptions involved in this procedure can be gained by further graphical analysis of the vector of residuals $\mathbf{Y} - \mathbf{X}\,\hat{\boldsymbol{\theta}}$. In Fig. 2 the residuals are plotted against $\mathbf{Y} = \mathbf{X}\,\hat{\boldsymbol{\theta}}$, the predicted average weights as suggested by Draper and Smith (1967). There is some indication that the variance of log W_i is not constant, as assumed. The transformation of variables resulting in the linear regression model 2 apparently overcompensates for the anticipated increase in the variance of W_i for larger fish. The result is that the observations on smaller fish receive a disproportionately high weight and the estimates, although still unbiassed, will not be the best.

METHOD 2

An alternative model that may be applicable under these circumstances is

$$W_i = \alpha L_i^{\beta} + \epsilon_i \tag{6}$$

in which the random error ϵ_i is additive. The model of equation 6 can be regarded as a nonlinear model for which application of the least squares criterion requires an iterative procedure. The residual sum of squares is defined as

$$S(\boldsymbol{\theta}) = \sum_i [W_i - \alpha L_i^{\beta}]^2 \tag{7}$$

and the least squares estimator $\hat{\boldsymbol{\theta}}$ is given by that value of the vector $\boldsymbol{\theta}$ which minimizes $S(\boldsymbol{\theta})$. If $E(\boldsymbol{\epsilon}) = \mathbf{0}$ the estimator will be unbiased and if, in addition, $\boldsymbol{\epsilon}$ is $N(\mathbf{0}, \sigma^2\,\mathbf{I})$, this estimator will be the same as the maximum likelihood estimator (see Draper and Smith, 1967).

To find the least squares estimator, equation 7 is differentiated with respect to $\boldsymbol{\theta}$, which provides normal equations of the form

$$\sum_i (W_i - \hat{\alpha} L_i^{\hat{\beta}})(-L_i^{\hat{\beta}}) = 0$$

and

$$\sum_i (W_i - \alpha L_i^{\hat{\beta}})(-\hat{\alpha} L_i^{\hat{\beta}}\, ln\, L_i) = 0$$

which are also nonlinear ,in the parameters.

A linearization technique described by Draper and Smith (1967), involving an approximation of equation 7, was used to obtain an iterated solution. This technique requires a preliminary estimate $\hat{\boldsymbol{\theta}}_0$ of $\boldsymbol{\theta}$, about which the model is expanded in a Taylor series terminated after the first order derivatives, as follows:

$$\hat{\alpha} L_i^{\hat{\beta}} \doteqdot \hat{\alpha} L_i^{\hat{\beta}}\Big|_{\hat{\alpha}_0, \hat{\beta}_0} + \frac{\partial(\hat{\alpha} L_i^{\hat{\beta}})}{\partial \alpha}\Big|_{\hat{\alpha}_0, \hat{\beta}_0} (\hat{\alpha} - \hat{\alpha}_0) + \frac{\partial(\hat{\alpha} L_i^{\hat{\beta}})}{\partial \beta}\Big|_{\hat{\alpha}_0, \hat{\beta}_0} (\hat{\beta} - \hat{\beta}_0)$$

so that, approximately

$$W_i - \hat{\alpha}_0 L_i^{\hat{\beta}_0} = L_i^{\hat{\beta}_0}(\hat{\alpha} - \hat{\alpha}_0) + \hat{\alpha}_0 L_i^{\hat{\beta}_0}\, ln\, L_i(\hat{\beta} - \hat{\beta}_0)$$

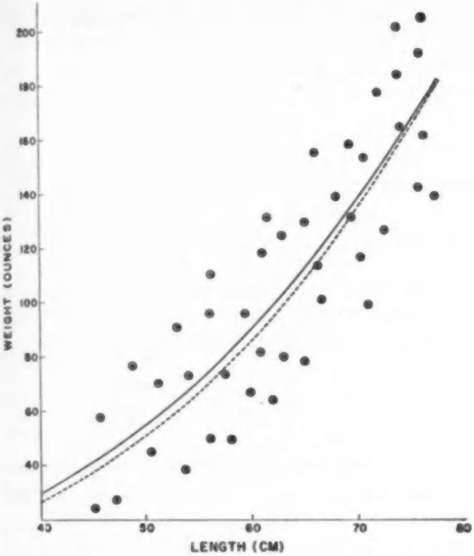

FIG. 1. Weight–length data for Pacific cod.

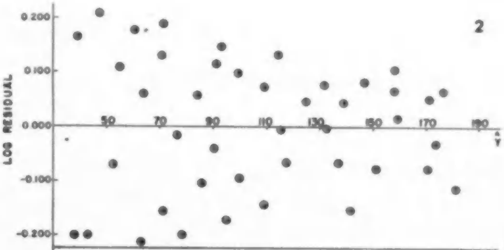

FIG. 2. Plot of log residuals in relation to predicted weight — method 1.

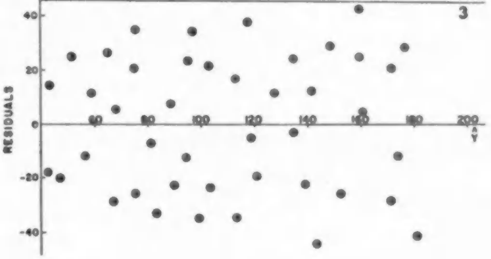

FIG. 3. Plot of residuals in relation to predicted weight — method 2.

or

$$Y_i = \sum_{j=1}^{2} \hat{\theta}_j X_{ji}$$

in an obvious notation, which is abbreviated to

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\theta} \tag{8}$$

in matrix notation. Equation 8 is of the standard linear form for which the least squares estimator is given by

$$\hat{\boldsymbol{\theta}} = (\mathbf{X}'\mathbf{X})^{-1}\ \mathbf{X}'\mathbf{Y}$$

as before, where

$$\hat{\theta}_i = \hat{\theta}_{i1} - \hat{\theta}_{i0} \qquad \text{for } i = 1,\ 2$$

so that

$$\hat{\theta}_{i1} = \hat{\theta}_i + \hat{\theta}_{i0}$$

is a revised estimate of θ_{i0}. This revised estimate is used in a further iteration until a convergent solution is obtained. In the present application of this technique, a convergent solution was assumed when in successive iterations j and $j + 1$.

$$\left|(\hat{\theta}_{i,\,j+1} - \hat{\theta}_{i,\,j})\ /\ \hat{\theta}_{i,\,j}\right| < \delta \qquad \text{for } i = 1, 2$$

where δ is an arbitrary small number. At each step in the iterative process, the residual sum of squares $S(\boldsymbol{\theta})$ was evaluated to demonstrate the actual reduction that was achieved.

This procedure yielded the following estimate of $\boldsymbol{\theta}$, using as $\hat{\boldsymbol{\theta}}_0$ the result obtained by method 1:

$$\hat{\boldsymbol{\theta}}' = [0.00130136,\ 2.72389].$$

The estimation equation becomes

$$\hat{W}_i = 0.00130136\ L_i^{2.72389}.$$

The fitted curve obtained by this method for the data of Table 1 is also included in Fig. 1 (solid line) for comparison with that obtained by the first method. As in the first instance, a plot of the residuals did not reveal a departure from normality but unlike the first instance, there is little, if any, indication of an unstable variance as shown in Fig. 3.

Another technique, commonly used in nonlinear estimation, involves the nonlinear normal equations and employs a similar approximation scheme, with the difference that the linearizing transformations are applied to the normal equations rather than to $S(\boldsymbol{\theta})$ (see, for example, Kempthorne, 1952).

The normal equations are given by setting $\partial S/\partial\theta = 0$, and are approximated by

$$\left.\frac{\partial S}{\partial \alpha}\right|_{\hat{\alpha},\hat{\beta}} = \left.\frac{\partial S}{\partial \alpha}\right|_{\hat{\alpha}_0,\hat{\beta}_0} + \left.\frac{\partial^2 S}{\partial \alpha^2}\right|_{\hat{\alpha}_0,\hat{\beta}_0}(\hat{\alpha}-\hat{\alpha}_0) + \left.\frac{\partial^2 S}{\partial \alpha\,\partial \beta}\right|_{\hat{\alpha}_0,\hat{\beta}_0}(\hat{\beta}-\hat{\beta}_0) = 0$$

and

$$\left.\frac{\partial S}{\partial \beta}\right|_{\hat{\alpha},\hat{\beta}} = \left.\frac{\partial S}{\partial \beta}\right|_{\hat{\alpha}_0,\hat{\beta}_0} + \left.\frac{\partial^2 S}{\partial \beta\,\partial \alpha}\right|_{\hat{\alpha}_0,\hat{\beta}_0}(\hat{\alpha}-\hat{\alpha}_0) + \left.\frac{\partial^2 S}{\partial \beta^2}\right|_{\hat{\alpha}_0,\hat{\beta}_0}(\hat{\beta}-\hat{\beta}_0) = 0$$

which are solved simultaneously for $(\hat{\alpha}-\hat{\alpha}_0)$ and $(\hat{\beta}-\hat{\beta}_0)$ to provide improved estimates of $\hat{\alpha}$ and $\hat{\beta}$, having started with initial estimates $\hat{\alpha}_0$ and $\hat{\beta}_0$. This technique gave identical results with the one that was used, but converged more slowly.

COMPARISON OF RESULTS AND COMMENTS

Estimates of average weight for different body lengths are presented in Table 2 for the two different methods. The total weight for the sample as estimated by method 2 is about 3% more than the weight estimated by method 1. To illustrate the practical significance of the difference it was assumed that a sample from a total catch of 1 million lb had a length distribution as shown in Table 3. This relative length frequency and the estimated average weights of Table 2 were used to estimate the total number of fish in the catch. The average weights estimated by the first method resulted in an estimate of 159,800 fish, compared with 154,350 for the second method, a difference of

TABLE 2. Estimated mean weights of Pacific cod.

Length (*cm*)	Weight 1[a] (*oz*)	Weight 2[a] (*oz*)	Weight 3[a] (*oz*)
40	26.7	30.1	27.8
45	37.6	41.4	39.1
50	51.0	55.2	53.0
55	67.3	71.6	70.0
60	86.7	90.8	89.9
65	109.4	112.9	113.8
70	135.7	138.1	141.1
75	165.9	166.7	172.5
80	200.1	198.7	208.1

[a] Weight 1 by method 1; weight 2 by method 2; weight 3 by method 1 with 4% positive correction.

TABLE 3. Hypothetical length frequency distribution for sample catch.

Length (*cm*)	% frequency	Estimated weight per 100 (1)	Estimated weight per 100 (2)
50	5	5×51.0	5×55.2
55	15	15×67.3	15×71.6
60	30	30×86.7	30×90.8
65	30	30×109.4	30×112.9
70	15	15×135.7	15×138.1
75	5	5×165.9	5×166.7
		10012.5	10366.0

over 3.4%. Length distributions with a smaller mean length will obviously lead to larger differences between the results, and vice versa.

It was interesting to note that the residual sum of squares ($S(\theta)$ of equation 7) for the second method was 26025.7, whereas for the first method it was 26495.2. At first glance, one may conclude that the second method, being superior in this respect, should therefore be preferred. This decision will depend largely on the extent to which we are willing to have the formulation of the model depend on the specific sample at hand. In the present example there is only a slight indication that the variance of W_i is increasing with length which can justifiably be ignored. In any event, the kind of weighting that resulted from the logarithmic transformation of the first method overcompensated for whatever increase in variance there may have been. It must also be pointed out that in the model used in the first method it is assumed that $\log W_i$ is symmetrically distributed around some mean value, which in effect results in nonuniform weighting across W_i with relatively more weight being assigned to the smaller fish weights within a length-class. If this seems unreasonable in the light of the information we have, a correction can be applied to predicted fish weights to correct for this nonuniform weighting. Meyer (1941) derived a correction factor which is a constant multiplier

$$m = 10^{1.1513\hat{\sigma}^2}$$

where $\hat{\sigma}^2$ is the residual variance as given by the first method. In our example $\hat{\sigma}^2 = 0.014885$, and therefore

$$m = 1.0403$$

which represents a correction of 4%. The predicted weights adjusted in this manner are also given in Table 2 for comparison. This procedure undercorrects at smaller sizes and overcorrects at larger sizes, relative to method 2.

It seems logical that the variance of W_i would be larger for larger fish and that the assumption of constant variance of the second method may not, in general, be justifiable. Even so, the specific weighting of the logarithmic transformation may overcompensate for the variance increase as in the present example. It may be necessary to deal with a model such as equation 6 in which $E(\boldsymbol{\epsilon\epsilon}') = \sigma^2 \mathbf{V}$ instead of $\sigma^2 \mathbf{I}$, where $\mathbf{V}$ is a diagonal matrix. If $\mathbf{V}$ were known and model 6 were premultiplied by a matrix $\mathbf{Z}$ with elements equal to the square root of the elements of $\mathbf{V}^{-1}$, then

$$\mathbf{ZW} = \mathbf{Z}\,\alpha X^{\beta} + \mathbf{Z}\boldsymbol{\epsilon}$$

and $$E(\mathbf{Z}\boldsymbol{\epsilon}) = \mathbf{0} \quad \text{if } E(\boldsymbol{\epsilon}) = \mathbf{0}$$

and also, $$E(\mathbf{Z}\boldsymbol{\epsilon\epsilon}'\mathbf{Z}') = \sigma^2\mathbf{I}.$$

In this manner a transformation of variables can be introduced which would satisfy the assumptions of regression analysis. For example, there may exist information to the effect that the variance of W_i is proportional to L_i, and the required transformation would alter equation 7 to

$$\sum_i \left\{ \left(\frac{1}{L_i}\right)^{1/2} \left[W_i - \hat{\alpha} L_i^{\hat{\beta}} \right] \right\}^2$$

from equation 6, which would become

$$\left(\frac{1}{L_i}^{1/2}\right) W_i = \alpha \left(\frac{1}{L_i}\right)^{1/2} L_i^{\beta} + \left(\frac{1}{L_i}\right)^{1/2} \epsilon_i.$$

In the present example, there is no obvious need for such a weighting procedure.

The common practice of using a linearizing transformation of the allometric weight–length relationship is not always justified in terms of the inherent assumptions, and can lead to considerable bias in the results. In cases where these assumptions seem unjustifiable, better estimates may be obtained by nonlinear estimation procedures which require a negligible amount of extra time and effort on today's high speed computers. A simple postmortem check on the justifiability of the assumptions is provided in Fig. 2 and 3 and the interested reader has reference to an extensive discussion of the examination of residuals by Anscombe (1961).

A computer program for method 2 has been written in Fortran (1130). The program will process up to 500 observations (as written) and will give both α and β, and the residuals of the observed data points. Source decks and listings can be obtained from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

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A Fry Trap Method for Estimating Salmonid Survival from Egg Deposition to Fry Emergence

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PHILLIPS, ROBERT W., AND K V. KOSKI. 1969. *A fry trap method for estimating salmonid survival from egg deposition to fry emergence.* J. Fish. Res. Bd. Canada 26: 133–141.

The method involves a trap of nylon netting placed over an individual redd with the trap's edges buried 15–20 cm in the gravel just outside the periphery of the redd. It has been used successfully on more than 70 coho salmon (*Oncorhynchus kisutch*) redds over the past 5 years, with as many as 2061 fry being captured from a single redd. The trap is relatively stable because it is flexible and conforms to the surface of the streambed, causing debris to float or roll over the surface. It can be used on individual redds; thus, emergent survival for separate parental combinations can be estimated. Field tests showed the efficiency of the trap approached 100%. Installation and presence of the trap had no significant effect on intragravel dissolved oxygen and gravel permeability. Mortality of fry in the traps averaged less than 1.5% when fry were removed at least three times a week. We concluded that the trap provides a more accurate estimate of survival from egg deposition through fry emergence than four other methods.

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INTRODUCTION

ADEQUATE METHODS for measuring survival of salmonid fishes from egg deposition to emergence of fry have not been available. This factor, which is essential to the understanding of the population dynamics of a stock, has usually been estimated from pre-emergent survival data or from weir counts of migrating fry. Trapping fry during their emergence is possible, and has many advantages over other means of estimating emergent survival. Several biologists have used rigid traps (Brasch, 1949; Hanavan and Skud, 1954; Heard, 1964; Gebhards, 1961; and J. A. R. Hamilton, Pacific Power & Light Co., Portland, Oregon, personal communication). In all cases, these consisted of wire or other mesh on a frame that extended above the water with the edges set several inches into the gravel around the redd. But the devices have not been widely used, being vulnerable to dislodgment by freshets. The purpose of this paper is to more fully describe a flexible trap (Phillips, 1966) that has proven successful because it conforms to the surface of the streambed.

DESCRIPTION OF TRAP

The fry trap is a cap of nylon netting placed over the redd with the edges buried to a depth of 15–20 cm around the periphery (Fig. 1). It is constructed from a piece of 3.2-mm mesh nylon netting, 2.4 × 4.6 m, with a tapered collection bag attached. The bag is 1.2 m long and is attached

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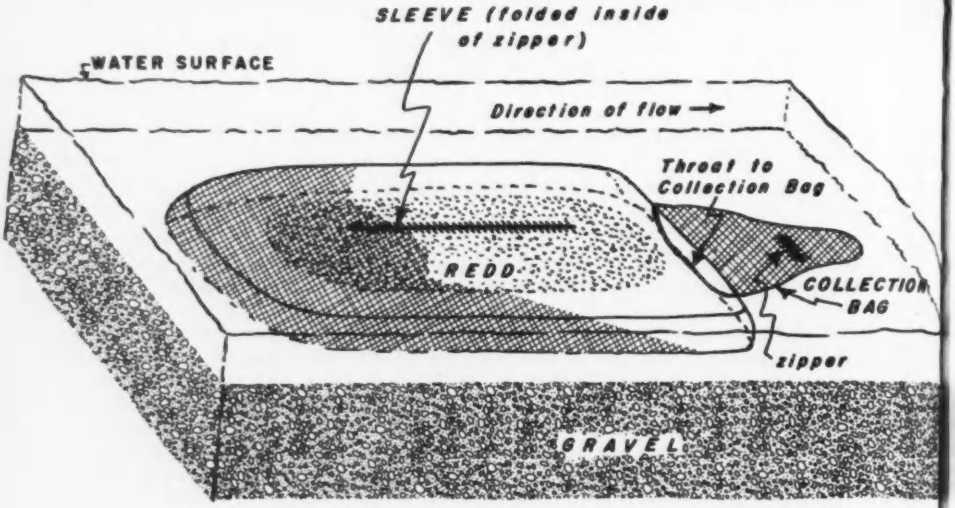
SLEEVE (folded inside of zipper)
WATER SURFACE
Direction of flow →
Throat to Collection Bag
REDD
COLLECTION BAG
zipper
GRAVEL

FIG. 1. Fry trap in place over a redd.

to the large rectangular piece of netting 0.3 m above the downstream end. Most fry are removed from the collection bag through a 0.6-m nickel zipper. A 1.5-m zipper is sewn into the center of the rectangular piece; it encloses a 0.9 × 1.5-m sleeve that, when extended (Fig. 2), provides access into the trap during its installation and for removal of fry.

The traps were constructed of nylon netting by sewing with a conventional sewing machine. Cost was about $50 including labor. The only other material tested was fiber glass screening, which proved unsatisfactory because it was too rigid in cold water.

The only unusual equipment needed to install the traps is six to eight sheet piles (Fig. 3), which consist of a driving point and socket welded to a 3-mm steel plate. They were fabricated at a cost of about $3.50 each. Other equipment includes a driving bar for the sheet piles, as well as a sledge hammer, a shovel, four or five buckets, and a small net. Shoulder-length neoprene gloves protect the hands and arms against the cold water.

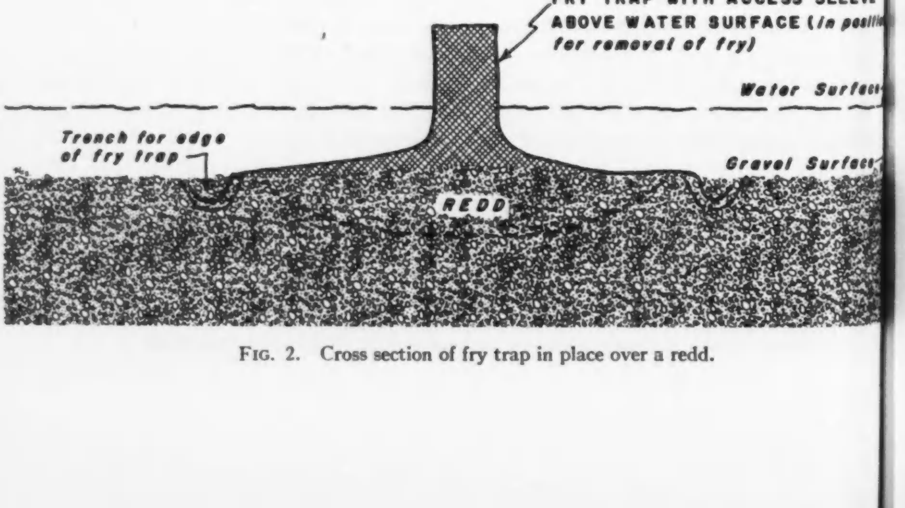
FRY TRAP WITH ACCESS SLEEVE ABOVE WATER SURFACE (in positi
for removal of fry)
Water Surface
Trench for edge of fry trap
Gravel Surface
REDD

FIG. 2. Cross section of fry trap in place over a redd.

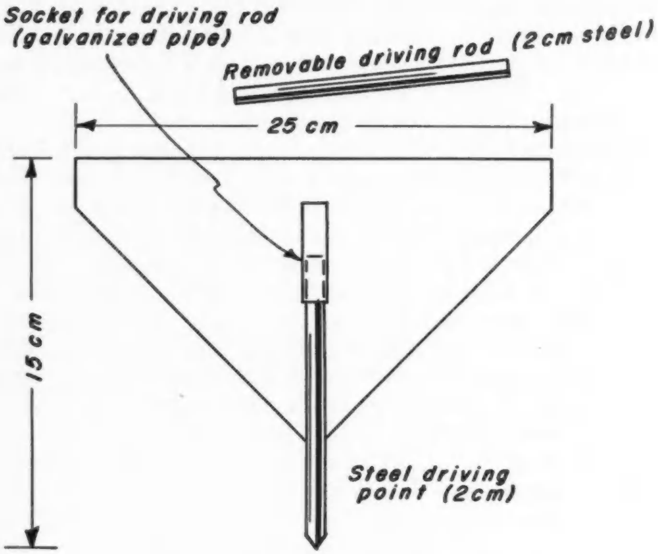

FIG. 3. Sheet pile for shoring side of trench.

Installation of a 2.4 × 4.6-m trap by two men requires 3–4 hr. The traps are durable and can be used for two or more seasons.

The boundaries of individual redds to be trapped are marked with stakes and flags immediately after spawning. Numbers of eggs deposited are estimated from lengths or weights obtained from females examined and tagged prior to spawning. Dates of eyeing, hatching, and emergence are estimated from temperature records. The traps are installed shortly before the estimated time of emergence.

At the time of installation, the nylon trap is spread over the redd, and a boundary for a trench is located and marked so as to leave a 15–30-cm strip of undisturbed gravel between redd and trench. Starting at the upstream edge of the redd, sheet piles are driven into the streambed in a single row to shore the redd side of the trench. A trench 15–20 cm deep is dug with a shovel along the outside of the sheet piles and the gravel placed in buckets. The net is placed immediately downstream to capture any alevins that might be released because of encroachment on the redd. The trap is then positioned so that about 30 cm of the border of the net hangs down into and across the bottom of the trench. Gravel from the buckets is replaced over the net in the trench. The sheet piles are removed and re-driven, and burial continued until the front and one side of the trap are installed. Driving the sheet piles and burying the netting proceeds along the opposite side until the entire redd is enclosed. Finishing at the downstream end allows the piles to be removed through the zipper on the collection bag. Redds in water deeper than about 0.5 m have been avoided because of the difficulty in digging the trench and burying the netting, but the use of diving equipment would make it possible to install traps in deep water.

Most of the fry emerging from the redd enter the collection bag and are easily removed. Brushing the trap is effective in driving fry into the bag. At the end of the emergence period an electric shocker and dipnet permit capture of the more elusive fry, which are removed through the sleeve or the collection bag.

EVALUATION OF TRAP

The trap has been used successfully since 1964 to capture coho salmon (*Oncorhynchus kisutch*) fry from individual redds in three small tributaries of Drift Creek in the Alsea River drainage on the Oregon coast. More than 70 redds have been trapped, and as many as 2061 fry captured from a single nest (Koski, MS, 1966). Mortality of fry in the trap averaged less than 1.5% when fry in the collection bag were removed at least three times a week. The trap has also been used successfully on cutthroat trout (*Salmo clarki*) redds at Drift Creek, on a chinook (*O. tshawytscha*) redd in the Carmen-Smith spawning channel on the McKenzie River in Oregon, and on a chum salmon (*O. keta*) redd at the University of Washington's Big Beef Creek fish research station.

Trap efficiency was tested by installing a 1.8 × 2.4-m trap and enclosing it with one measuring 2.4 × 3.7 m (Fig. 4). A standpipe was buried inside the inner trap with a release chamber 25 cm below the gravel surface. Fry normally emerge from the gravel upon absorbing their yolk sacs. For these tests, there was still a little yolk remaining when fry were released into the gravel. In 1966, one standpipe was buried in the middle of the inner trap and 100 fry were released into the gravel. In 1967, a more rigorous test of the trap was made with four evenly spaced standpipes inside the inner trap, each installed about

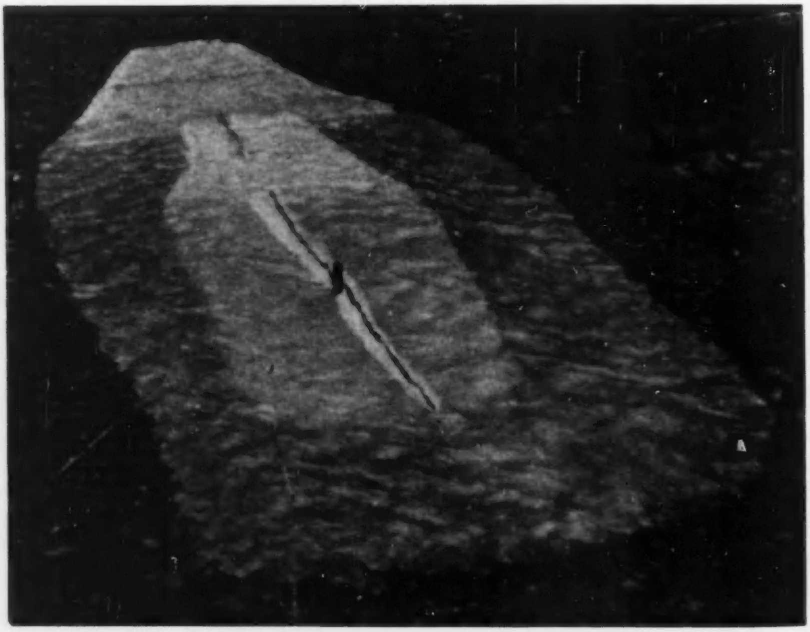

FIG. 4. Fry traps used in efficiency tests. Test trap is enclosed by larger trap. Fry are introduced into gravel by means of standpipe in center.

1 ft from its edge. About 200–300 alevins were equally allocated to the standpipes in each test trap. In both years, efficiency was determined by dividing the number of fry recovered inside the inner trap by the total number recovered inside both traps.

The tests show (Table 1) the trap was almost 100% efficient in capturing fry emerging from a redd when used as described in the streams where we were working. Actual test efficiencies were probably greater than shown since several of the fry taken in the outer trap, because of their greater size, were suspected of entering through the open zipper when the investigators were removing fry from the inner trap. On the other hand, we assumed that if fry emerged beyond the inner trap, most of them would be captured by the outer trap. The number that actually emerged outside both traps is unknown, but it is believed to be small for the reasons discussed in the following paragraphs.

TABLE 1. Summary of efficiency tests of fry trap at Drift Creek, Alsea River, Oregon.

Year	Test trap	No. of fry released into gravel	Emergence (%) Inner trap	Emergence (%) Outer trap	Efficiency (%)
1966	1	100	8	0	100
	2	100	45	0	100
	3	100	76	1	99
	4	100	0	0	—[a]
	5	100	26	0	100
	6	100	0	0	—[a]
1967	7	198	14	0	100
	8	200	42	2	97
	9	200	34	0	100
	10	300	74	0	100
	11	300	64	0	100
	12	275	35	0	100
					Mean = 99.6

[a] These data were not included in determining mean percentage of efficiency.

Emerging fry could elude the trap only by moving laterally through the gravel. Such movement may be extensive in large, uniform gravel but it would be difficult if not impossible in gravel containing a high proportion of fine material, such as we found in the undisturbed area between redd and trench. Also, experiments in the laboratory indicated that coho fry emerging from a point 25 cm below the gravel surface undertake little lateral movement. In gravel whose composition was similar to that found in coho redds, 78% emerged within a 23 cm radius of the point of burial. This was determined by setting a fiber glass screen directly above the eggs. The edge of the screen was buried

10 cm into the gravel to reduce escapement of fry after they had emerged by their reentering the gravel, perhaps to emerge at another point.

Two other investigators reported on the distribution of fry during, and prior to, emergence. Stuart (1953) found that brown trout (*S. trutta*) initially moved horizontally, then upward, dispersing as they moved in a manner to make the pattern of emergence somewhat cone-shaped with the apex toward the point of origin. McNeil (1964) studied the distribution of pink salmon (*O. gorbuscha*) eggs and alevins in the gravel, and found little indication that they dispersed horizontally in redds prior to emergence.

For the fry trap to measure natural survival, it must have no effect on the environment. Tests were therefore run by means of standpipes to determine whether disturbance of gravels in installing the trap would influence the intragravel environment and hence the developing alevins. A *t*-test indicated no significant difference ($P = .05$) between the mean dissolved oxygen concentrations before and after the traps were installed on 25 coho redds (Koski, MS, 1966). A similar test also indicated no significant difference ($P = .05$) in gravel permeability before and after the traps were installed. These results show that installation and presence of the trap has little effect on the intragravel environment.

Silt has a greater tendency to settle on the gravel inside the trap than outside because of reduced water velocity in the enclosed area. A scrub brush was effective in removing it by swirling the water on the trap surface. The brush was also used to dislodge algae that attached to the netting.

The trap is relatively stable in the stream. Because it lies close to the gravel surface, debris usually floats or rolls over it. The device resists dislodging by freshets. For example, an increase in stream discharge from 0.4 m^3/sec at the time of installation to 2.4 m^3/sec later in the season caused no dislodgment or damage.

COMPARISON WITH OTHER METHODS

Trapping of fry at the time of emergence has certain advantages over other methods of estimating survival. Often, such estimates have been made by excavating redds and determining the ratio of live eggs and alevins to the total number found (Hobbs, 1937; Briggs, 1953; and many others). Disappearance of eggs and alevins by gravel movement, predation, scavenging, and decomposition, however, would cause these survival estimates to be too high (Carl, 1940; Shaw and Maga, 1943; McDonald, 1960; McNeil, 1964). Survival estimates made by the use of fry traps are not biased in this way since they are made by dividing the number of fry captured by the estimated number of eggs deposited. However, fry trap estimates are subject to the error inherent in estimating the number of eggs in a female from her length or weight, because of eggs that might have been lost in prior spawning or because of eggs retained in the body cavity. Care must be taken, of course, not to include redds of other females inside the trapped area.

Estimates of survival have also been based on the number of live eggs and alevins recovered from a porous container (Gangmark and Broad, 1955; Coble, 1961; and Phillips and Campbell, 1962). This method has the advantage of starting with a known number of eggs, but the containers would exclude most predators and scavengers, and result in unrealistically high estimates. The fry trap also errs in this manner because it, too, would tend to exclude predators such as sculpins (Hikita and Nagasawa, 1960; McLarney, MS, 1964; Phillips and Claire, 1966) and large carnivorous stonefly larvae (Stuart, 1953; Claire and Phillips, 1968).

Estimates of alevin density in a spawning area just prior to emergence largely prevent the over-estimation of survival from disappearance of eggs and alevins (McNeil, 1964). However, the sampling plan proposed by McNeil when flushing alevins from gravel works best where there is a relatively high density of eggs deposited in a spawning area such as is typical in pink and chum salmon spawning beds. This method, as well as the preceding two that are compared with the fry trap, is deficient in that it does not take into account mortality during emergence. Small gravel (Shelton, 1955) and increases in the proportion of fine particles in gravel (Koski, MS, 1966) have been shown to decrease survival, apparently by blocking emergence. Several unpublished experiments by the Oregon State Game Commission also have shown an increasing rate of fry entrapment with increasing percentages of fine particles in the gravel. Confinement within the gravel bed can thus prevent fry from emerging, causing survival estimates based on flushing out or digging of fry to be too high. The trap captures fry at the redd area *after* emergence; thus, mortality caused by confining gravel has been accounted for.

The trap is unsuited for estimating survival at intermediate times between egg deposition and fry emergence, and is not as good for this determination as the three methods discussed above. McNeil's (1964) method is especially good for this purpose.

Survival estimates based on weir counts might be realistic approximations for salmonids that leave their natal stream immediately after emerging (Pritchard, 1947; Foerster and Ricker, 1953; Hunter, 1959; Merreill, 1962; and Wickett, 1962). Any predation losses from the time of emergence to capture at the weir, however, would cause these estimates to be too low (Hunter, 1959). The fry trap would not be similarly biased because it protects the newly emerged fry from most predators. Of course, care must be taken not to enclose such animals inside the fry trap when installing it.

CONCLUSION

We believe the trap provides a more accurate estimate of salmonid survival from egg deposition through fry emergence than other methods. It also has the advantage of being used on individual redds; survival for separate parental combinations can be estimated. It probably has little adverse effect on behavior of emergent fry since mortality is low if fry are removed from the collection bag at least three times a week, and migration from the redd need only be delayed for a short time. The trap is now being used in before-and-after studies to measure the impact of changes in the intragravel environment caused by logging.

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NOTES

Observations on the Greenland Shark, *Somniosus microcephalus*, in Northern Baffin Island

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BECK, B., AND A. W. MANSFIELD. 1969. *Observations on the Greenland shark, Somniosus microcephalus, in northern Baffin Island.* J. Fish. Res. Bd. Canada 26: 143–145.

Eighteen immature Greenland sharks (*Somniosus microcephalus* (Bloch and Schneider) 1801) were captured during the course of a study on the biology of the narwhal, *Monodon monoceros*. Most had been feeding on offal resulting from the whaling operation and on fish and seal meat discarded at the campsite. Other food consumed included two egg cases of the arctic skate, *Raja hyperborea*, previously unrecorded from this locality. Four narwhals and one white whale were attacked in the nets, the sharks removing large chunks of skin and blubber from the carcasses.

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IN THE COURSE OF a study on the biology of the narwhal, *Monodon monoceros*, carried out in northern Baffin Island during the summers of 1963, 1964, and 1965, 18 Greenland sharks (*Somniosus microcephalus* (Bloch and Schneider) 1801) were captured. The first specimen was taken by seal harpoon when it nosed onto the beach in front of our camp in Koluktoo Bay (72°06′N, 80°47′W), but the remaining 17 specimens were taken from the 18-inch (45-cm) mesh nets used for catching the narwhals. Nine male sharks ranged in size from 237 to 311 cm (tip of rostrum to extreme end of tail) and were all immature, judging from histological examination of the testes. Eight females ranged in size from 217 to 295 cm and were also immature since no visible eggs were present in the ovaries. Sharks of similar size (7–10 ft; 214–305 cm), and presumably immature, have been taken in previous years at Pond Inlet (Mary-Rousselière, 1959), but no adult specimens have been reported from northern Baffin Island.

Though apparently a deepwater species, the Greenland shark is often attracted to surface waters where offal from sealing or whaling operations is disposed of (Hansen, 1953; Templeman, 1963, p. 36–42). Undoubtedly the sharks caught in our nets at Koluktoo Bay were attracted by the offal resulting from our whaling operations. Besides the remains of narwhals, a number of unwanted carcasses of ringed seals, *Pusa hispida*, and some arctic char, *Salvelinus alpinus*, were thrown into the sea near the nets. The sharks not only ate much of this waste, but they attacked the whales caught in the nets as well

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(Table 1). From a total of 58 narwhals and 1 white whale netted in Koluktoo Bay, 4 narwhals and the white whale were attacked by sharks before being examined by us. At each netting station, at least one whale had been caught in the preceding week and processed on the beach, the waste from which provided an attractant to sharks.

TABLE 1. Stomach contents of 17 Greenland sharks taken in Koluktoo Bay, northern Baffin Island.

Field no.	Date	Sex	Length (*cm*)	Stomach contents
SM 1	Aug. 22, 1963	M	270	Seal meat, char
3	Aug. 21, 1964	F	261	Amphipod, brittle star, 2 egg cases of *Raja hyperborea*
4	"	F	285	Small unidentified fish
5	"	F	271	Char
6	"	F	217	Amphipod, gastropod, shark offal
7	"	M	297	Amphipod, sea urchin, gastropod (minus shell), kelp, char, narwhal offal, sealskin
8	Aug. 27, 1964	F	295	Char
9	"	M	237	Char, narwhal offal
10	Aug. 28, 1964	M	269	Gastropods
11	Aug. 29, 1964	F	260	Char, seal
12	"	M	302	Unidentifiable
13	"	F	237	Empty
14	Aug. 30, 1964	M	311	Seaweed
15	"	F	220	Char
16	"	M	270	Empty
17	"	M	310	Unidentifiable
18	"	M	254	Char

In spite of an unimpressive array of teeth (Fig. 1) the Greenland shark is able to remove large chunks of skin and blubber from a whale carcass. The smooth semicircular nature of the bites made by upper and lower jaws and the precise way in which skin, and blubber are removed suggest that the shark is a slow feeder and bites very deliberately (Fig. 2, 3).

Though offal was the principal food consumed, six stomachs were found to contain benthic organisms. One of these (SM 3) contained two egg cases of the arctic skate, *Raja hyperborea*, a species which has been recorded by Jensen (1948) from near Pond Inlet and to the northward on the Canadian side of Baffin Bay.

The parasitic copepod *Ommatokoita elongata* (Grant) was found attached to the cornea of several specimens. None of our sharks had a copepod attached to each eye, as Berland (1961) found in 1270 sharks (84.4%) taken in East Greenland waters. The external appearance of this parasite is shown in Fig. 4. According to Berland (1961) the round scars scattered over the cornea are

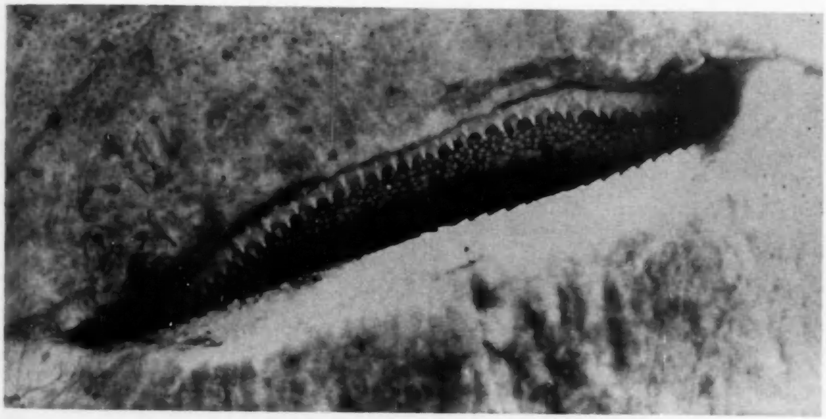

FIG. 1. Upper and lower teeth rows of the Greenland shark.

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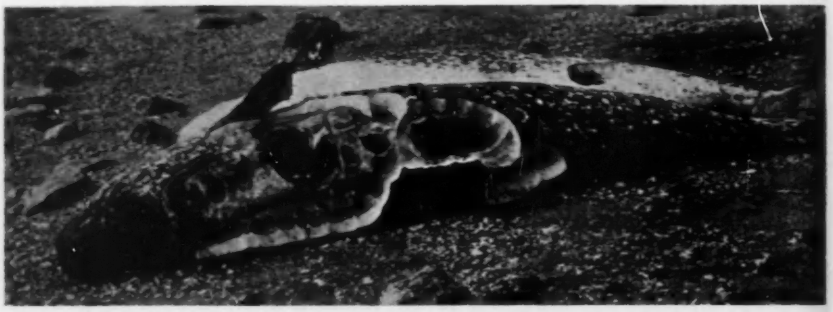

FIG. 2. Narwhals attacked by Greenland sharks after being captured in nets.

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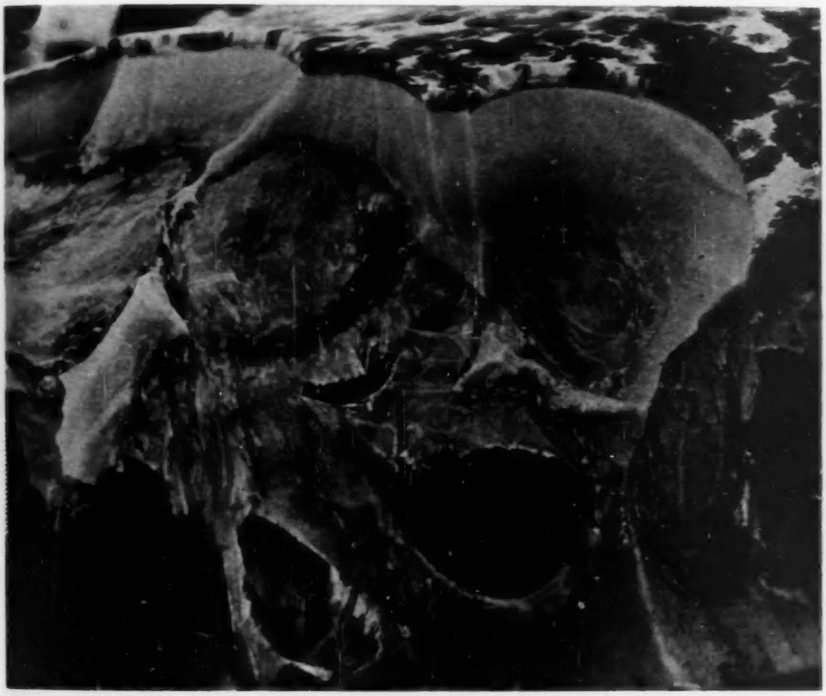

FIG. 3. Closeup view of dorsal skin and blubber of nearer whale in Fig. 2.

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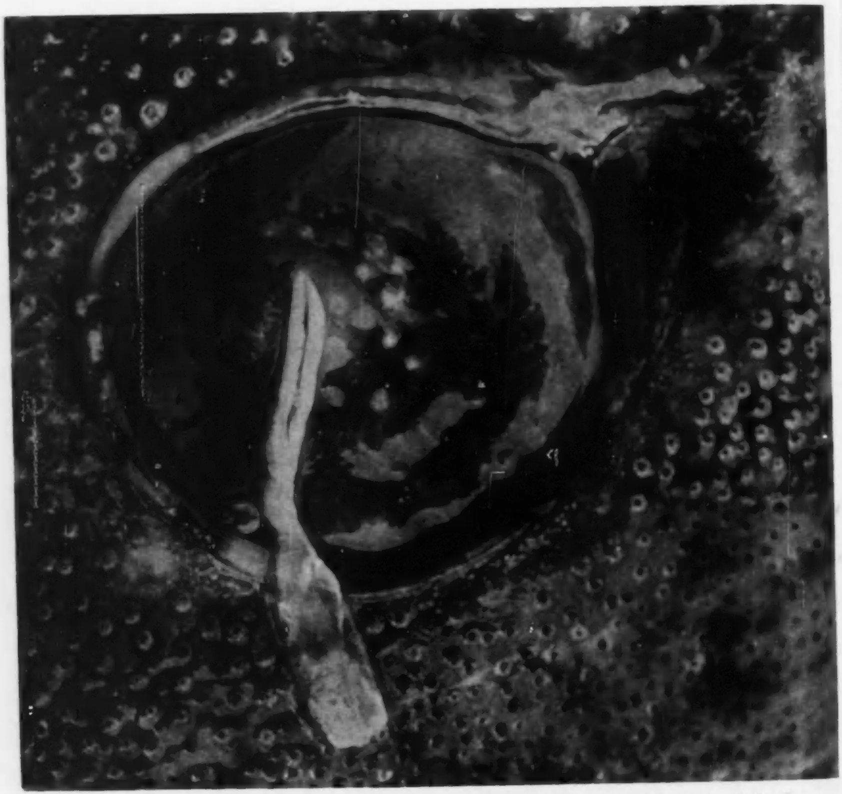

FIG. 4. Parasitic copepod *Ommatokoita elongata* attached to eye of Greenland shark.

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probably the sites of former attachment of the parasite. Sharks with two or more parasites attached to one eye are rare.

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Estimates of the Zinc Requirements of Marine Organisms

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The amount of enzyme-bound zinc is calculated from estimates of total enzyme concentrations and the number of zinc atoms per mole of these enzymes. This estimate is shown to be smaller than measured concentrations in marine organisms, indicating that zinc is accumulated in excess of the organisms' immediate needs. A minimum "potential fertility" for zinc is calculated; the results indicate that zinc is not limiting in the marine environment.

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ZINC IN BIOLOGICAL SYSTEMS apparently is required only as a constituent of several oxidation–reduction, hydrolytic, and lytic enzymes (Dixon and Webb, 1964; Bonner and Varner, 1965). Many marine organisms can concentrate zinc from ambient waters (Rice, 1963; Polikarpov, 1966), and it is of interest to determine whether the zinc concentrations in the organisms are needed immediately to satisfy metabolic requirements, or are in excess of their needs. Marine animals probably obtain their enzyme-bound zinc by consuming other animals or plants, or their remains, whereas marine plants undoubtedly accumulate the element from sea water. If zinc is assumed to be the limiting element for marine plants, it should be possible to calculate the maximum amount of plant tissue, or carbon, which could be synthesized from sea water containing a given amount of zinc. Such a calculation would be similar to Harvey's (1947) calculations of "potential fertility" of sea water on the basis of phosphorus and nitrogen concentrations.

In this note we (i) calculate the amount of zinc needed in biological systems by estimating quantities of total enzymes and the number of zinc atoms per mole of these enzymes; (ii) compare the required zinc concentrations with those actually measured in marine plants and animals; and (iii) estimate the potential fertility of sea water with measured concentrations of zinc.

For a first approximation of the moles of enzymes (moles$_e$) in a kilogram of soft tissue (kg$_t$), Srere (1967) assumed that approximately 20% of soft animal tissue is composed of enzymatic protein (*e*). We assumed that the 20% estimate was adequate for soft plant tissue as well as for that of animals; even if the estimate is tripled, however, the conclusions to be drawn are not affected. Srere (1967) further assumed that enzymes have an average molecular weight of 10^5 g/mole. The average molecular weight applies to known zinc-containing enzymes (Dixon and Webb, 1964). We now calculate

$$\frac{200\ \text{g}_e/\text{kg}_t}{10^5\ \text{g}_e/\text{mole}_e} = 2 \times 10^{-3}\ \text{mole}_e/\text{kg}_t.$$

Eleven of the 829 enzymes (1.33%) listed by Dixon and Webb (1964) contain zinc. Assuming Dixon and Webb's list to be representative, then out of any large number of enzymes, 1.33% will contain zinc. Because several of the zinc-containing enzymes have more than one zinc atom per molecule (e.g., alcohol dehydrogenase has two or four zinc atoms/molecule, and D-lactate dehydrogenase has three), a ratio of 17 moles per 829 moles of enzyme is probably more accurate. We then calculate a zinc requirement per unit weight of tissue:

$$\frac{17\ \text{moles}_{\text{Zn}}}{829\ \text{moles}_e} \times 2 \times 10^{-3}\ \text{mole}_e/\text{kg}_t = 4.1 \times 10^{-5}\ \text{mole}_{\text{Zn}}/\text{kg}_t.$$

Then

$$4.1 \times 10^{-5}\ \text{mole}_{\text{Zn}}/\text{kg}_t \times 65.4\ \text{g}_{\text{Zn}}/\text{mole}_{\text{Zn}} = 2.7 \times 10^{-3}\ \text{g}_{\text{Zn}}/\text{kg}_t = 2.7\ \text{ppm}.$$

Because the estimate of enzyme concentration was for soft tissues only, the value of 2.7 ppm is high, on a total live (wet) weight basis, for organisms with calcareous or siliceous exoskeletons. Therefore, 2.7 ppm probably represents a maximum zinc requirement for live marine organisms.

Measured concentrations of zinc in marine plants and animals vary considerably among organisms (Table 1), but are in all cases much higher than the calculated zinc requirement. Only benthic algae (seaweeds) were

Table 1. Measured concentrations of zinc in marine plants and animals.

Organism	ppm Zn (*wet wt*)	Method[a]	Reference
Marine algae			
Pelvetia canaliculata	21	WC	Black and Mitchell (1952)
Ascophyllum nodosum	20	"	"
Fucus vesiculosus	34	"	"
Nereocystis luetkeana	29[b]	"	Wort (1955)
Marine animals			
Coelenterata			
Deep-sea anemone	24[b]	AAS	A. G. Carey[c]
Echinodermata			
Pisaster ochraceous	64[b]	"	"
Benthopecten sp.	47	"	"
Hymenaster sp.	144[b]	"	"
Thrissascanthias penicillatus	213	"	"
Paleopatides sp.	15	"	"
Abyssicucumis albatrossi	48[b]	"	"
Mollusca			
Bathydoris sp.	158	AAS	"
Crassostrea virginica	2040[d]	ES	Alexander and Rowland (1966)
Crassostrea virginica	1330[b]	WC	Chipman et al. (1958)
Mytilus edulis	81[e]	P	Macchi (1965)
Chiroteuthis sp.	16	NA	W. O. Forster[c]
Gonatus sp.	19	NA	"
Arthropoda (Crustacea)			
Gammarid amphipod	14	NA	"
Sergestes similis	11	NA	"
Sergestes similis	12[b]	AAS	W. G. Pearcy[c]
Calanus sp.	41	"	"
Thysanoessa spinifera	20	"	"
Euphausia pacifica	14	"	"
Euphausia pacifica	30	"	S. W. Fowler[c]
" muscle	26	"	"
" molts	360	"	"
Cancer magister	566	"	A. G. Carey[c]
" muscle	45	"	"
" shell	7	"	"
Carcinus maenas	25[b]	WC	Bryan (1966)
Homarus vulgaris	22[b]	WC	"
Mitella polymerus — body	300[d]	ES	Alexander and Rowland (1966)
Mitella polymerus — stem	54[d]	ES	"
Chordata (Pisces)			
Merluccius merluccius	15[e]	P	Macchi (1965)
Merluccius sp.	23	AAS	W. G. Pearcy[c]
Engraulis mordax	24[b]	"	"
Thaleichthys pacifica	16[b]	"	"
Diaphus theta	13[b]	"	"
Diaphus theta	12	NA	W. O. Forster[c]
Microstomus pacifica	10	"	"
Argyropelecus lychnus	27	"	"
Scomber scombrus	155	ES	Noddack and Noddack (1940)
Squalus acanthias	420	ES	"
Mixed zooplankton	57	NA	W. O. Forster[c]

[a] WC, wet chemistry; NA, neutron activation analysis; AAS, atomic absorption spectrometry; ES, emission spectroscopy; P, polarography.

[b] Average of several determinations.

[c] Unpublished data.

[d] Assuming a dry-to-live-weight ratio of 0.2.

[e] Assuming an ash-to-live-weight ratio of 0.03.

included for plants in Table 1 because, to our knowledge, there have been no direct measurements of stable zinc in phytoplanktonic species in the ocean. However, there is evidence from radiotracer experiments that phytoplankton accumulate zinc in excess of their needs. Boroughs et al. (1957) demonstrated that cells of the marine diatoms *Nitzschia* were capable of dividing 10 times following depletion of zinc from the water. Accumulation of zinc in marine plants is probably by nonmetabolic adsorption–exchange (Gutknecht, 1965; Broda et al., 1964), but can be related indirectly to metabolism (Gutknecht, 1961). Recently Cross et al. (1968) and S. W. Fowler (unpublished data) have shown in autoradiographs that ^{65}Zn accumulated from both food and water by small marine crustaceans was associated almost entirely with surfaces of hard and soft tissue. It was not incorporated in significant amounts into soft tissue over a period of 14 days. Also, the specific stable zinc concentrations in just-molted exoskeletons of *Euphausia pacifica* contained almost 14 times the zinc associated with muscle tissue from the same individuals on a live weight basis (Table 1). Such evidence suggests that adsorption–exchange is the major mechanism for zinc concentration in marine crustaceans, and perhaps in other marine animals. Any of several functional groups found in proteins, polysaccharides, and nucleic acids might be sites of zinc accumulation (Schubert, 1954).

Expressing zinc accumulation by marine plants as "potential fertility" indicates that zinc is not a limiting element in the sea. We define potential fertility on the basis of zinc, after Redfield et al. (1963), as the quantity of carbon which could be produced by photosynthesis from a unit volume of sea water if it were brought from depth to the surface and illuminated there until the zinc was exhausted. Even though total measurable zinc in sea water has been separated into ionic and nonionic fractions (Rona et al., 1962; Torti, 1967; Kečkeš and Pučar, 1966), several workers have indicated that there is an equilibrium between the fractions (Torti 1967; Keckeš and Pucar, 1966). Although ionic zinc might be the only form incorporated by organisms, nonionic zinc might act as a reservoir. As a first approximation, we can calculate potential fertility assuming that all zinc in the sea is available to organisms. Using an average total zinc concentration in the sea (Goldberg, 1963) of 10 μg/liter (0.15 $\mu\text{mole}_{\text{Zn}}$/liter), and our estimated concentration in soft tissue (4.1×10^{-8} $\text{mole}_{\text{Zn}}/\text{g}_l$), we calculate

$$\frac{0.15\ \mu\text{mole}_{\text{Zn}}/\text{liter}}{0.041\ \mu\text{mole}_{\text{Zn}}/\text{g}_l} = 3.7\ \text{g}_l/\text{liter}.$$

Assuming a dry weight to live weight ratio of 0.2 for soft tissues, and further assuming that half of the dry weight is carbon (C) (Redfield et al., 1963), we then calculate the potential fertility as:

$$0.2 \times 0.5 \times 3.7 = 0.37\ \text{gC/liter} = 370\ \text{gC/m}^3.$$

A recalculation of potential fertility using the lowest concentration of ionic zinc (0.6 μg/liter) reported by Rona et al. (1962) yields a value of 22 gC/m^3. Redfield et al. (1963) set the potential fertility on the basis of nitrogen in the sea at 2.74 gC/m^3, a value considerably lower than our potential fertility estimates for either total or ionic zinc. These calcuations indicate that zinc would not be limiting in the marine environment.

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Culture of Calanoid Copepods in Synthetic Sea Water

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The calanoid copepods *Acartia tonsa* and *Eurytemora affinis* were successfully cultured in two commercial synthetic sea salts. Success of a medium was measured by its ability to sustain a population of copepods that was subjected to moderate rates of harvest.

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RECENT REPORTS have demonstrated that calanoid copepods can be cultured in natural sea water (Jacobs, 1961; Zillioux and Wilson, 1966; Corkett, 1967; Mullin and Brooks, 1967). The use of sea water, particularly of neritic origin, as a culture medium has disadvantages. Although organic material can be eliminated by filtration and by photo-oxidation (Armstrong et al., 1966; Hamilton and Carlucci, 1966), the resulting product has a somewhat variable composition. To persons located far from the sea, cost is an additional disadvantage. In an attempt to overcome these problems I tested two commercially produced, synthetic sea salts as culture media.

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During the course of a study of copepod production, the calanoid copepod *Eurytemora affinis* (Poppe) was grown with moderate success in dilute solutions of both "RILA Marine Mix" (RILA Products, P.O. Box 114, Teaneck, New Jersey, USA 07666) with 25 mg sodium bicarbonate added per liter, and "Instant Ocean" (Aquarium Systems, Inc., 1450 East 289 St., Wickliffe, Ohio, USA 44092). Excellent results were obtained with another calanoid, *Acartia tonsa* (Dana), in Instant Ocean (Table 1). Numerous attempts at growing *A. tonsa* in RILA Marine Mix failed.

TABLE 1. Summary of exploited laboratory cultures of copepods: BW = filtered bay water, IO = "Instant Ocean," RILA = "RILA Marine Mix."

Culture no.	Species	Temp. (*C*)	Total time culture was harvested (*days*)	Total no. of times harvested	Culture medium
1	*Acartia tonsa*[a]	20	260	65	BW
2	"	20	161	45	IO
3	"	20	183	43	BW
4	*Eurytemora affinis*	15	179	30	BW
5	"	20	216	51	BW
6	"	20 & 15	227	45	IO
7	"	20	106	21	RILA
8	"	20	62	15	RILA + NaHCO$_3$ (25 mg/liter)
9	"	20	22	6	RILA + NaHCO$_3$ + vitamins[b]

[a] The *A. tonsa* in culture 1 were obtained from the Patuxent estuary 1 year later than the *A. tonsa* in cultures 2 and 3.

[b] The vitamin mix was the same as that used in the algal culture medium.

The criterion used to measure success of a medium was the persistence of a population of copepods subjected to a modest harvest rate. At selected intervals (3–4 days at 20 C, and 5–7 days at 15 C), the cultures were harvested. They were stirred vigorously and one half of their volume was poured out and was concentrated in a Clarke-Bumpus net cup fitted with a No. 62 Nitex net window. The concentrated material was preserved in formalin and counted as yield.

The copepods used were obtained from the Patuxent River estuary, Maryland, in the winter of 1965–66. Prior to starting cultures in synthetic sea water, they were grown in water from Chesapeake Bay that was filtered through No. 1 Whatman filter paper and then diluted to a salinity of 12‰. The synthetic sea water was also diluted to 12‰ with distilled water. Cultures in filtered bay water served as controls for evaluating the synthetic media (Table 1).

A mixture of three algal species was fed to the copepods. They were *Chaetoceros* sp., *Chlamydomonas reinhardti* (Dangeard) (Indiana University Strain 90), and *Isochrysis galbana* (Parke). The cultures of *Chaetoceros* were contaminated with at least one species of bacteria, but axenic stocks of the other two algae were maintained. The cultures of copepods were not axenic. The algae were grown in 125-ml cotton-stoppered flasks in a synthetic medium made by adding the following to 1 liter of distilled water: 12 g RILA Marine Mix, 25.5 mg $NaHCO_3$, 50.0 mg $NaNO_3$, 5.0 mg Na_2HPO_4, 15.0 mg $NaSiO_3$, 0.5 mg Fe (as Sequestrene NaFe), 2.86 mg H_3BO_3, 0.2 mg Thiamin HCl, 0.001 mg Biotin, and 0.001 mg Vitamin B_{12}.

The copepods were fed three times a week with algae that had attained optical densities that varied from 0.080 to 0.130 at a wavelength of 550 $m\mu$. Approximately 80–90% of the optical density was due to *I. galbana*. Packed cell volume and algal cells per ml were directly proportional to optical density. At an optical density of 0.090, packed cell volume was 0.004–0.005 ml per 10 ml of algal culture. The mixture of algae was provided at a rate of 12.5 ml per liter of copepod culture at each feeding. Other organisms in the copepod cultures may also have been eaten by the copepods. Two rotifer species and several protozoans were present in numbers sufficient to be a source of trace nutrients.

Cultures of copepods were maintained at constant temperature (15 or 20 C) and under continuous light in 0.5- or 2.0-liter Erlenmeyer flasks. The flasks were covered with black polypropylene to reduce the growth of epiphytes, but light was not totally excluded. Cultures grown in filtered bay water were transferred to clean flasks periodically because previous experience indicated that the coating of bacteria and blue–green algae that accumulated on the sides and bottom of the flasks was associated with a cessation of egg laying by the copepods. This problem occurred also in RILA Marine Mix but not in Instant Ocean. A coating formed in the flasks of Instant Ocean but the blue–green algae were absent. A rust colored precipitate always formed in the Instant Ocean and stained the sides of the flasks. The rust color was also prominent in the copepod fecal pellets.

The ratio of the length of time that a culture was harvested to the number of harvests provided a rough measure of success (Table 1). The only culture in a synthetic medium that produced as well as the cultures in bay water was *A. tonsa* in Instant Ocean (Table 1, culture 2). Although *E. affinis* was cultured longer in synthetic media, the numbers of copepods present varied more than in the cultures of *A. tonsa*. Rapid decreases in the density of *E. affinis* in synthetic media were always the result of reproductive failure. Egg sacs, normally carried by the female until the nauplii hatch, were dropped prematurely. The sacs were sometimes intact, and sometimes broken. This difficulty seldom occurred in filtered bay water. Separate experiments with individual females indicated that the fraction of the dropped eggs that hatched ranged from 0 to 100% and was roughly proportional to the fraction of the normal development time that the eggs were carried by the females. Because 20 C and 12‰ salinity may be outside the optimum temperature–salinity combination for *E. affinis* (Heinle, MS, 1965), a species that is sometimes found in fresh water (Northcote et al., 1964), the temperature of the culture in Instant Ocean was lowered to 15 C during the experiment. The population maintained itself at a low density although subjected to only modest harvest (Table 1, culture 6). The culture in bay water at 15 C flourished (Table 1, culture 4).

Early in this work, mortalities of entire cultures occurred frequently. I believe that detergent residues on the glassware caused these problems since mass mortalities were eliminated by cleaning the glassware with strong acid.

The results shown in Table 1 suggest that Instant Ocean is a suitable culture medium for *A. tonsa*. Although *E. affinis* was grown for 15 months in artificial media, reproductive success continued to be poor, possibly the result of a nutrient deficiency or a toxic effect.

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Low-Temperature Fish Storage Facility with Precise Temperature Control

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Scott, K. R. 1969. *Low-temperature fish storage facility with precise temperature control.* J. Fish. Res. Bd. Canada 26: 154–161.

A coldroom complex recently installed at the Fisheries Research Board of Canada, Freshwater Institute laboratory at Winnipeg features several design aspects that are considered novel. These include foamed-in-place urethane insulation, two alternating R-502 refrigeration systems incorporating automatic safety switch-over and adjustable defrost, "straight-line" pneumatic temperature control, hot gas bypass control, and a master panel. The facility combines a cold laboratory at +2 C, a long-term storage room at –37 C, a small anteroom at –26 C, and a room containing eight 10.0-ft^3 precise temperature cabinets at –40 C. Room temperature variation is ±0.25 degrees C during steady state conditions. Temperature rise during daily defrosting is less than 2 degrees C for a duration of 1 hr.

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Previous investigations by Dyer et al. (1957) on the keeping quality of frozen fish indicate the need not only for low temperature but also for accurate temperature control during storage life. After the Freshwater Institute of the Fisheries Research Board of Canada was established at Winnipeg in 1966, a survey was made of laboratory coldrooms to determine the optimum configuration. The following general arrangement was selected for the installation at the Freshwater Institute: (1) a cold laboratory at +2 C having 176 ft^2 (16.4 m^2) of floor area, a stainless steel laboratory bench 16 ft (4.88 m) long complete with sink, water, distilled water, storage drawers, and electrical receptacles (Fig. 1B); (2) a dormant storage area at –37 C having 112 ft^2 (10.4 m^2) of floor area (Fig. 1C); (3) a precise temperature storage area at –40 C for controlled experiments having 109 ft^2 (10.1 m^2) of floor area (Fig. 1D); (4) an anteroom to the precise temperature room at –26 C having 40 ft^2 (3.7 m^2) of floor area (Fig. 1E).

Layout of rooms— Space considerations dictated combining the cold laboratory and the coldrooms into an integrated suite of rooms with an adjoining equipment room for the refrigeration apparatus (Fig. 2). The rooms (Fig. 1A) are of free-standing, prefabricated, insulated panel construction for ease of installation and to permit expansion or relocation at a later date. The exterior finish is baked-on white enamel; the interior is embossed stucco aluminum and

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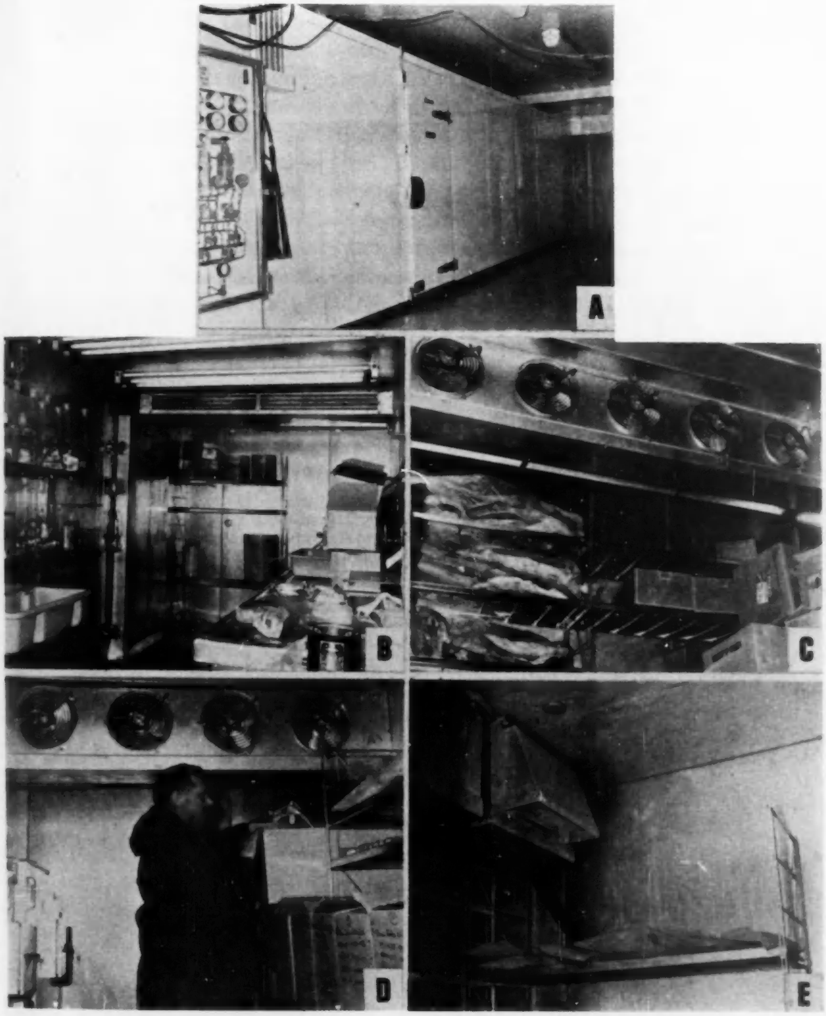


FIG. 1. (A) Exterior view; (B) cold laboratory, room 1; (C) cold room, room 2; (D) cold room, room 3; (E) anteroom, room 4.

the floors are 0.040-inch (0.00102-m) stainless steel over $\frac{3}{4}$-inch (0.019-m) plywood with a bearing capacity of 200 lb/ft^2 (976 kg/m^2). All floor joints are sealed with silver solder to eliminate contamination and facilitate cleaning. The walls, ceiling, floor, and interior doors are insulated with 4 inches (0.102 m) of foamed-in-place polyurethane with a K factor of 0.11 PCU/ft/hr/ (degrees C/inch) (i.e., 0.137 g cal/cm/hr/degrees C). Door gaskets consist of heavy sponge rubber, and mating hard rubber ribbed extrusions to eliminate

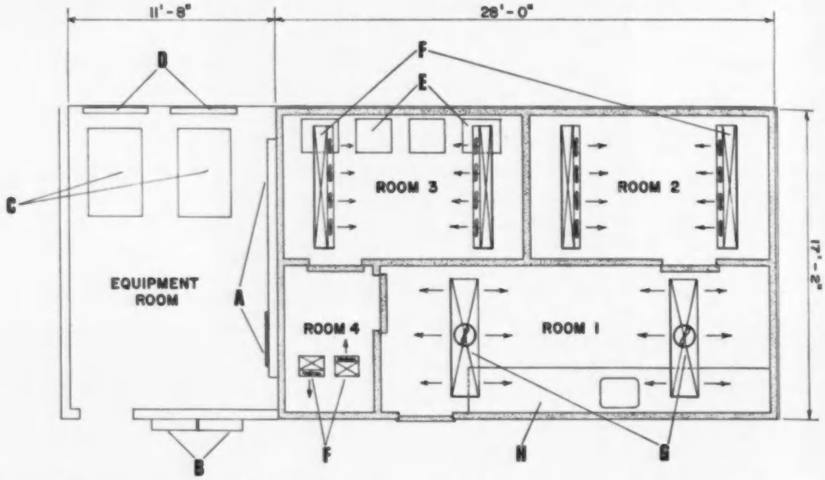

11'-8"
28'-0"
D
F
E
C
ROOM 3
ROOM 2
EQUIPMENT ROOM
A
ROOM 4
ROOM 1
17'-2"
B
F
H
G

FIG. 2. Room layout: (A) operating control panel; (B) master control panel; (C) hermetic refrigeration compressor; (D) re-evaporator; (E) precise temperature cabinets; (F) cooling coils with hot gas defrost; (G) low velocity cooling coil; (H) laboratory bench.

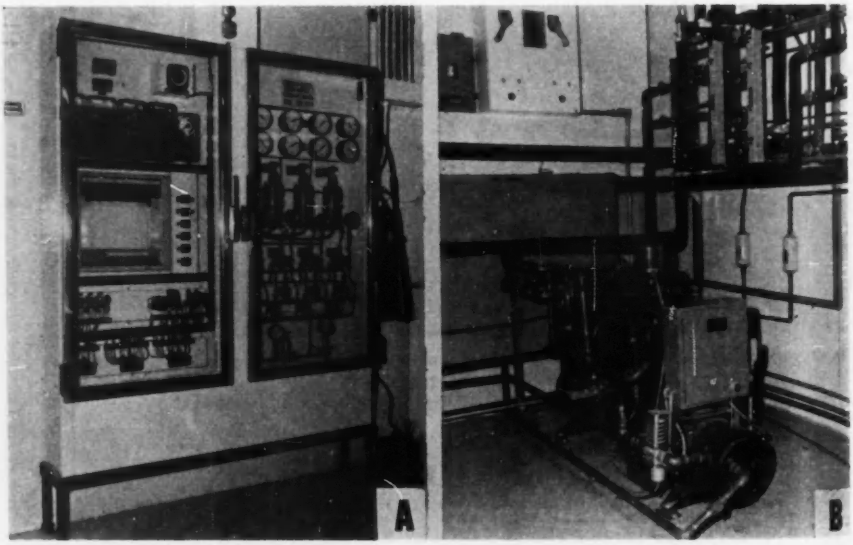


FIG. 3. (A) Master control panel; (B) hermetic compressor.

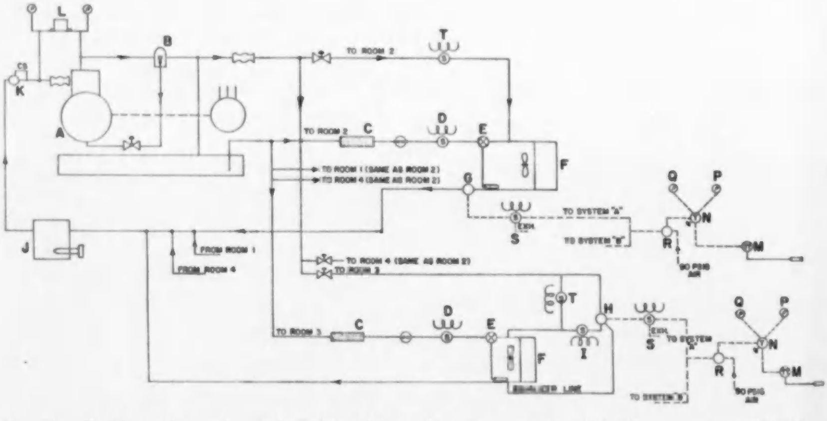
frost buildup on the edges. The exterior cold laboratory door is of lightweight construction having 2.5 inches (0.0635 m) of urethane insulation and is equipped with a foot treadle to streamline traffic flow. To prevent frost buildup, care was taken to ensure tight joints between all panels. Electrical fixtures are vapor-proof with conduits passing through panel walls carefully caulked inside and outside. Free-standing stainless steel adjustable shelves of modular construction designed for free air flow are provided for general storage of fishery products. In room 3, six 10.0-ft^3 (0.283-m^3) constant temperature cabinets controlled by SCR (silicon controlled rectifier) temperature controllers permit rigid control of temperatures from –40 C to 0 C. The cold laboratory, room 1, is equipped with vapor-proof fluorescent light fixtures giving a lighting level of 100 ft-c (1076.4 lumens/m^2).

Refrigeration system— All four rooms are cooled by one 10-hp single-stage water-cooled hermetic compressor (Fig. 3B) using refrigerant R-502 and having a 20-hp motor for fast temperature pulldown. A second complete system with no refrigerant piping interconnection provides standby in the event of trouble with the operating system. The total cooling load for the four rooms is 10,281 pound-calorie units (PCU)/hr, apportioned as follows:

Room & temp	Wall load	Air changes	Occupancy	Lights	Constant temp cabinets	Total
1 (+2 C)	790	1210	350	1045		3395
2 (–37 C)	1062	481	145	189		1877
3 (–40 C)	1117	239	145	189	2670	4360
4 (–26 C)	593			56		649
Grand total						10281

Allowing for pressure losses between the coil and the suction of the compressor, a condensing unit with a capacity of 14,750 PCU/hr at –48 C furnishes 43% excess capacity for quick temperature recovery. The draw-through cooling coils (Fig. 2F), in the three cold rooms are sized for 2.8 degrees C temperature difference between room temperature and refrigerant saturation temperature to maintain high room humidity. Defrosting is by hot gas, controlled through solenoid valves located on the operating control panel (Fig. 2, A). Baffles on the inlet and outlet of the coil minimize air circulation and hence temperature deviation during defrosting. Coils are equipped with continuously energized drain line heaters to prevent refreezing of removed frost. The cold laboratory coils (Fig. 4, G) are not equipped for defrosting since room temperature is above freezing. These coils are designed for 5.5 degrees C temperature difference and are of special low profile, low velocity design with air entering the bottoms of the coils and discharging horizontally from both sides at ceiling level. This arrangement reduces drafts and adds greatly to the comfort of working personnel.

Liquid refrigerant is metered to the cooling coils (Fig. 4, F) by thermostatic expansion valves (Fig. 4, E) and liquid solenoid valves (Fig. 4, D). Temperature control in the various rooms is by a combination of a hot gas bypass control and evaporator pressure regulators. Room 3, which is held at the coldest temperature (–40 C), uses a hot gas bypass control system with pneumatic pilot control. This system imposes an artificial load on the refrigeration compressor raising the suction pressure until a balance is achieved between system capacity and room heat gains. At this point corrective adjustments cease and the room is at the temperature desired. Since this temperature, and hence suction pressure, is the lowest desired in any of the rooms it is therefore possible to control the temperature in all the other rooms by the simple application of evaporator pressure regulators, also equipped with pneumatic pilot control, operating from impulse signals coming from the control system. In addition to this basic control system, a minimum low temperature limit in any of the rooms is imposed by manual adjustment of the range springs in the evaporator pressure regulators or hot gas regulator. This safety feature operates in the event of failure of the control air supply and is particularly useful for the cold laboratory (room 1) where temperature must not fall below 0 C to prevent freezing.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N
TO SYSTEM "A"
EXH
S
TO SYSTEM "B"
R
M
90 PSIG AIR
J
FROM ROOM 1
FROM ROOM 4
TO ROOM 4 (SAME AS ROOM 2)
TO ROOM 3
T
H
D
E
TO ROOM 3
C
F
I
S
EXH
TO SYSTEM "A"
Q
P
N
R
M
EQUALIZER LINE
TO SYSTEM "B"
90 PSIG AIR

FIG. 4. Refrigeration schematic: (A) refrigeration compressor; (B) oil separator; (C) filter-dryer; (D) liquid solenoid valve; (E) thermostatic expansion valve; (F) evaporator cooling coil; (G) evaporator pressure regulator; (H) hot gas bypass regulator; (I) hot gas regulator solenoid; (J) electrical re-evaporator; (K) crankcase regulator; (L) combination high–low pressure switch; (M) remote temperature transmitter; (N) pneumatic temperature control; (P) control point dial gauge; (Q) room temperature dial gauge; (R) pneumatic amplifier relay; (S) control line solenoid; (T) hot gas solenoid.

Pneumatic controls— Operating controls are mounted in a master control panel equipped with lockable glass doors (Fig. 3A).

The four rooms are equipped with pneumatic temperature transmitters (Fig. 4, M) connected to adjustable temperature controls (Fig. 4, N) having a range of –40 to +4.4 C. Two 3$\frac{3}{4}$-inch (9.5-cm) diam dials (Fig. 4, P, Q) for each of the four rooms indicate respectively the control point temperature and the actual room temperature. The modulating pneumatic temperature controls are two mode controllers having separate adjustments for proportional band (sensitivity) and reset rate adjustment. This permits balancing refrigeration capacity to system characteristics for each individual room. A continuous comparison is made between the air impulse from the room temperature transmitter and the desired control point. The output control pressure to the final control elements (Fig. 4, G, H) is thus constantly adjusted, resulting in "straight line" temperature control for all rooms (Fig. 5). Pneumatic amplifier relays (Fig. 4, R) between the controller output port and the final control elements boost the air impulse pressures to the range of coil saturation pressures for R-502 which will produce the desired room temperatures. The pneumatic control system is common to both operating and standby units, the appropriate impulse lines to the final control elements being selected by control line solenoids (Fig. 4, S) operated by the automatic switchover system. Separate safety high-

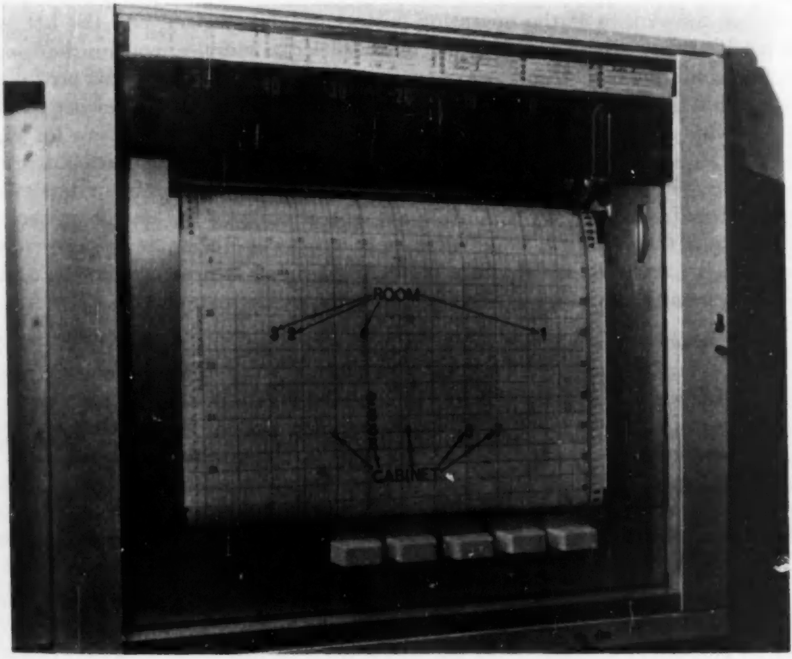

FIG. 5. Strip chart temperature records.

limit thermostats, also connected to the switchover system, are installed in all four rooms.

Electrical controls— At any given time only one refrigeration compressor is operating. The standby unit, however, will automatically be energized through a pressure cut-in switch (Fig. 4, L) to ensure maintenance of temperatures if the temperature in any of the four rooms exceeds an independently adjustable high temperature setting, or if the operating compressor fails because of high pressure cut-out, low oil pressure cut-out, winding high limit thermostat cut-out, control transformer failure, control curcuit overload, line fuse failure, or magnetic starter thermal overload trip-out.

Simultaneously a manual-reset bell and lamp circuit are energized. Amber pilot lights on the control panel indicate the operating compressor. A time clock provides automatic alternation of compressors to keep total running times approximately equal. A push button allows for manual compressor transfer. For room 2 a special, manually operated rapid-pulldown timer and relay by-passes the main control sequence and enables both cooling coils to operate simultaneously. This minimizes the extent and the duration of the set-point deviation during periods of high room loading.

Defrosting of the evaporator coils is controlled automatically by a separate timer for each of the four rooms. During the defrost cycle the liquid line solenoids and coil fan motors of the operating system are deenergized and the hot gas solenoids are energized allowing the coils to defrost. Simultaneously, the liquid line solenoids and coil fan motors of the standby system are energized providing interim refrigeration to minimize temperature fluctuations. The defrost timers also provide a fan start delay at the end of the defrost period to allow for adequate drainage of water from the coils after defrosting. An additional relay keeps the standby cooling coils operating for 15 min after defrost thus minimizing room temperature rize while the operating coils are are being cooled below room temperature. Switchover does not occur during defrosting as the sequence is controlled by separate relays. Additional indicating pilot lights in the equipment room (Fig. 2) show which coils are defrosting.

Discussion— Temperatures in all four rooms can be held within ±0.25 degree C (Fig. 5) except during daily defrosting when the temperature rises 2 degrees C for about 1 hr. These variations are measured with copper–constantan thermocouples (24 AWG) which, because of their small thermal mass, are very sensitive and have a short time-constant. The variation shown on the room temperature dials during the defrost cycle is only about 1 degree C and, since control action is in response to deviation of the temperature dials from the control point, the selected proportional-band setting is as narrow as possible without producing cycling during steady state conditions. The precise-temperature cabinets control air temperatures to ±0.5 degree C even during defrosting. Actual product temperature variation would be much less than this because of the short duration of the deviation and the large thermal mass of the stored products.

One minor difficulty, which was encountered in room 1 (+2 C), was a temporary drop in temperature below freezing during loading of fish into room 2 (-37 C). This difficulty was expected and could have been minimized by the addition of an anteroom; however, space considerations prevented this arrangement.

The pneumatic feedback control system performs reliably and effectively, the individual proportional band and reset adjustments providing an easy method of offsetting any imbalance between system capacity and room heat gain rates. One necessary precaution is to instal a refrigerated instrument-air aftercooler on the pneumatic compressor if control air lines pass through cold areas such as room 1. This is necessary to prevent condensate from freezing due to Joule–Thompson expansion as the air passes through the control orifices in the pneumatic transmitters. If the pneumatic transmitters are to be mounted on a cold wall they should also have a thermal insulation barrier to minimize heat conduction to the wall.

The cost of the entire installation including erection and start-up was $43,000. The system described has operated for over a year with no major problems or breakdowns and has provided reliable, accurate low-temperature control for research on fishery products.

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Comparison between Gompertz and von Bertalanffy Curves for Expressing Growth in Weight of Fishes

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The two types of curves were fitted to empirical weights-at-age for four commercially important species: sardines, haddock, lake trout, and chum salmon. For the range of observed data, both curves fit the empirical points well, and seem almost indistinguishable on this basis. The upper asymptotic limit of weight, however, was higher for the von Bertalanffy than for the Gompertz, in all four examples. Fitting complexity is similar for both types of curves, but the Gompertz does not require the assumption of cubic length–weight relation implicit in the Beverton–Holt treatment of the von Bertalanffy.

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IN MY POPULATION MODELING by analog computer (Silliman, 1967), I had occasion to use the Gompertz (1825) growth curve, which at the time I believed to be simpler for analog computation than the von Bertalanffy curve (1938). Although this view has since been discredited (Richards, 1968), it still seems useful to make a comparison between the two types of curves.

The von Bertalanffy curve gained attention in the work of Beverton and Holt (1957) and its subsequent widespread use and analysis among fishery biologists (Taylor, 1962; Chapman, 1960; Paulik and Gales, 1964; Abramson, 1965; Allen, 1966; Tomlinson and Abramson, 1961; Southward and Chapman, 1965). The Gompertz curve has also been used for aquatic organisms (Weymouth and McMillin, 1931; Hile, 1943; Silliman, 1967) and has been subjected to competent biometric analysis as a growth curve (Winsor, 1932).

The mathematical expressions for the two curves (both can be derived from the general differential equation for growth given by Chapman, 1960) are:

$$\text{Gompertz } W_t = W_r\, e^{G-Ge^{-g(t-t_r)}}$$

$$\text{von Bertalanffy } W_t = W_\infty \left[1-e^{-K(t-t_0)}\right]^3$$

where t = age of fish in years; t_r = age of fish at recruitment to commercially fished stock; W_t = weight of fish at age t; W_r = weight of fish at recruitment; W_∞ = asymptotic upper limit of weight; t_0 = age of fish at zero weight; G, g, and K = empirically determined constants.

The above expressions reveal aspects of the curves which could revive interest in the Gompertz curve:

1. The von Bertalanffy curve (as treated by Beverton and Holt) was first applied to *length*, and then to *weight* by assuming a simple cubic relation. It has been demonstrated repeatedly by numerous investigators that the exponent differs substantially from three in many fishes — see Paulik and Gales (1964) for recent comments on this subject.

2. The Gompertz curve may in some situations be simpler to combine with other exponential expressions (such as those for mortality) than the von Bertalanffy curve.

3. Both curves require the calculation of three constants (G, g, and W_r for the Gompertz; K, t_0, and W_∞ for the von Bertalanffy), so that fitting complexities should be similar.

To compare the two curves, I selected representatives from commercially important groups of fishes: *pelagic* — Pacific sardine (*Sardinops sagax*); *demersal* — Atlantic cod (*Gadus morhua*) and Atlantic haddock (*Melanogrammus aeglefinus*); *freshwater* — lake trout (*Salvelinus namaycush*); *anadromous* — chum salmon (*Oncorhynchus keta*).

All empirical weights-at-age were obtained from the published works cited in Table 1, and were fitted by the method of Beverton and Holt (1957) for

TABLE 1. Data for Gompertz and von Bertalanffy fits to weight-at-age data. Symbols: for Gompertz t_r = age curve begins; W_r = weight at t_r; G, g = empirical constants; for von Bertalanffy: t_0 = age at zero weight; K = empirical constant; for both: W_∞ = asymptotic weight; r^2 = coefficient of determination, regression of fitted curve on empirical values.

Item	Pacific sardine, *Sardinops sagax*	Atlantic cod, *Gadus morhua*	Haddock, *Melanogrammus aeglefinus*	Lake trout, *Salvelinus namaycush*	Chum salmon, *Oncorhynchus keta*
Source of length or wt-at-age data	Phillips, 1948	Schroeder, 1930	Wise, 1957	Van Oosten, 1950	Ricker, 1964
Source of length–wt relation	Clark, 1928	Bigelow and Schroeder, 1953	Wise, 1957; Silliman and Wise, 1961	–	Ricker, 1964
Length–wt expt.[a]	3.15	3.08	1.81	–	3.20
Wt units	Grams	Pounds	Pounds	Pounds	Kilograms
Ages inclusive	2–9	1.5–7.5	1–9	2–8	1–4
Gompertz data:					
t_r	0	0	0	2	1
W_r	28.02	0.4433	0.1203	0.2475	0.2706
G	2.015	4.500	3.549	3.415	3.784
g	0.469	0.220	0.386	0.410	0.370
W_∞	210.2	39.9	4.18	7.53	11.90[b]
r^2	0.999	0.997	0.997	0.992	0.998
von Bertalanffy data:					
t_0	−1.58	−0.75	−1.125	0.719	−0.19
K	0.40	0.16	0.265	0.224	0.242
W_∞	213.8	44.0	4.66	11.25	13.35[b]
r^2	0.999	0.996	0.998	0.985	0.998

[a] This is x in the expression weight = C (length)^x.

[b] These constants have no biological meaning for the chum salmon; this asymptote would not be closely approached until several years beyond the maximum age of the species (all chum salmon die after spawning at an age of 5 years or less). For instance, an individual following the Gompertz curve would not achieve a weight of 11.4 kg (within 0.5 kg of the asymptote) until age 13.

the von Bertalanffy curve and by the analog computer technique of Silliman (1967) for the Gompertz curve. Results are summarized in Table 1. The values of r^2 (coefficient of determination for regression, fitted on empirical points) show that both types of curves provide excellent fits within the range of observations and account for 98.5–99.9% of the variation in the empirical data. There seems to be little basis for choice here.

The Gompertz does differ from the von Bertalanffy curve, however, in the values of W_∞. These values are higher for the latter than for the former in all of the examples. Thus extrapolation beyond the range of empirical points, as is sometimes necessary in population simulation, would lead to higher average weights for the von Bertalanffy than for the Gompertz curve. General knowledge might provide a basis for choice.

In summary, four points seem pertinent: (1) each of the curves requires the calculation of three constants; (2) the use of the simple cubic length-weight relation in the Beverton–Holt application of the von Bertalanffy curve may be open to objection and is not necessary for the Gompertz curve; (3) both curves provide excellent fits to weight-at-age of typical representatives of four groups of commercially important fishes, within the ranges of observation; (4) the upper asymptotic limit of weight was higher for the von Bertalanffy than for the Gompertz curve in all of the examples.

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Gill Dimensions as a Function of Fish Size[1]

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The relationship between total gill area and body weight can be expressed as $Y + aW^b$ where "*b*" varies from about 0.8 to 0.9 for different species. The area for a 1-g fish ("*a*") ranges 22-fold for published data on different species. Expressed by the same general equation, the number of secondary lamellae per millimeter of filament ranges 4-fold for different species and decreases with increasing fish weight within each species by a power of about –0.1.

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Attempts have been made to correlate estimates of respiratory surface area in fish with "activity" (e.g. Gray, 1954; Hughes, 1966) and to utilize published estimates in models of growth and metabolism (e.g. Brown, 1965; Ursin, 1967) and gas exchange (e.g. Hughes, 1966; D. J. Randall, personal communication). Such studies all point to the need for an explicit statement of the effect of fish size on the gill dimensions. Because gill area per gram of fish decreases with increasing fish size, valid comparisons of different species have been restricted to fish of similar size. Estimates of gill area for different species are available for fish ranging from less than 1 g to about 40 kg so the restriction is not desirable and it should not be necessary.

This note examines some of the existing data and attempts to demonstrate the effect of fish size on the total respiratory surface area and on the number of secondary lamellae per millimeter of filament (the inverse of which indicates the "fineness" of the "sieve" or water channels). The hypotheses being considered

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[1] Contribution 126 from the Bedford Institute.

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are that gill area is proportional to fish weight and that the number of secondary lamellae per millimeter of filament is constant for all sizes of a given species. The effect of fish size on the dimensions of individual secondary lamellae, the total number of filaments, total filament length, etc., have been considered by Price (1931) and by Muir and Hughes (in preparation) and are not included here for the data are lacking in many of the other studies.

The data from Price (1931) for bass range from less than 1 g to nearly 1 kg, and those from Muir and Hughes (Gill dimensions for three species of tuna) (in preparation) for tuna from 1 to 40 kg. Those for other species involve short size ranges and often only one size (Table 1). Gray's (1954) measurements are supplemented by additional data of his published by Hughes (1966). The data of Byczkowska-Smyk (1957, 1958, 1959) were omitted for Hughes (1966) states that her values may be in error. Dubale's (1951) measurements of the gill areas for 41 species are not comparable for they involve only the total area of the filaments and not of the secondary lamellae.

The total lamellar area for all species is plotted (log–log) against fish weight in Fig. 1. The four prominent lines (labelled) without points are lines of best fit obtained by regression analysis and have the following slopes:

Skipjack tuna	0.85	Muir and Hughes (in prep.)
Yellow and bluefin tuna	0.90	Muir and Hughes (in prep.)
Gray's intermediates	0.82	Ursin (1967)
Bass	0.78	Price (1931)

All of the other lines simply join points for different sizes of fish belonging to the designated species. The lower broken line, indicated by a query, suggests a possible lower limit on the basis of the existing measurements. Reference lines, with slopes of 1.0 and 0.8, are shown at the lower right. The logarithmic transformation of $Y + aW^b$ adequately linearizes the data for most species, and for many the value for the slope, "b," is in the neighborhood of 0.8. Hughes (personal communication) reports values of about 0.9 for trout and roach. The value for carp (46) is about 1 and those for *Trachurus* (32) and *Callionymus* (45) appear to be greater than 1. Excepting these, the gill area for fish weighing 1 g ("a" in the above equation) may range from about 2.5 to 54 cm², the largest being about 22 times the smallest. Comparison of gill areas for fish of 1000 g avoids the need to except these species and eliminates the possible errors in extrapolation. At this size the areas range from 700 to 18,500 cm² or 26-fold.

Gray (1954) and Hughes (1966) showed tabular correlations between gill area/g and the "activity" of each species. Such correlation is evident in gross in Fig. 1, but biochemical factors which vary from species to species undoubtedly play an important role in the relationship between gill area and metabolism.

It is of interest to attempt a comparison of mammalian lung areas with the gill areas reported for fish. Values obtained from standard text books range from 10 to 90 m² for the lung surface in man but 70 m² appears most often (e.g. Comroe, 1965, p. 15). Whereas gill areas are obtained by direct measurement, lung areas can be estimated by indirect methods only and may not carry the

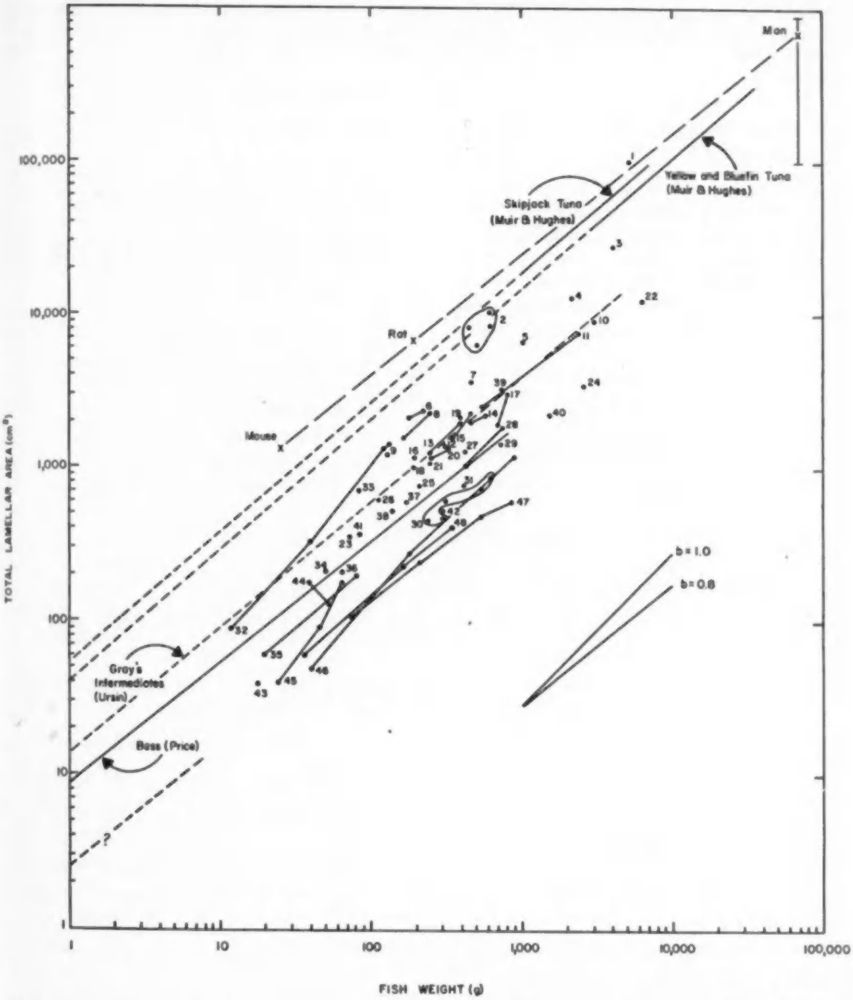

FIG. 1. Relationship between fish weight and the total lamellar area for various species of fish. Numbers refer to list in Table 1. See text.

same precision. In neither case, however, is there much certainty of the portion of the surface area that is functional in the transfer of gases. Hughes (1963, p. 3) gives 54 and 33 cm^2/g as the lung surface areas for white mouse and white rat. Assuming a weight of 25 g for the mouse and 200 g for the rat (and 70 kg for man) the appropriate values were plotted in Fig. 1. These few data suggest that the effect of animal weight on respiratory surface area may be similar in fish and mammals and that the magnitude of the area of the gill surface of tuna approaches that of the lung surface in mammals.

Table 1. Literature sources and species of fish used in the comparative study of gill dimensions. Numbers identify species shown in Fig. 1 and 2. The scientific names used by the original authors are retained and common names of the species studied by Hughes (1966) are added for consistency.

Gray's measurements (Gray, 1954, and Hughes, 1966)

1. *Gymnosarda alleterata*, false albacore
2. *Brevoortia tyrannus*, menhaden
3. *Coryphaena hippurus*, dolphin
4. *Sarda sarda*, bonito
5. *Pomatomus saltatrix*, bluefish
6. *Scomber scombrus*, common mackerel
7. *Scomberomorus maculatus*, spanish mackerel
8. *Mugil cephalus*, jumping mullet
9. *Caranx crysos*, hard tail
10. *Roccus lineatus*, striped bass
11. *Archosargus probatocephalus* sheepshead,
12. *Chilomycterus schoepfi*, bur fish
13. *Stenotomus chrysops*, scup
14. *Tautoga onitus*, tautog
15. *Prionotus strigatus*, red-winged sea robin
16. *Poronotus triacanthus*, butterfish
17. *Cynoscion regalis*, sea trout
18. *Palinurichthyes perciformis*, rudderfish
19. *Echeneis naucrates*, remora
20. *Spheroides maculatus*, puffer
21. *Centropristis striatus*, sea bass
22. *Lophius piscatorius*, goosefish
23. *Peprilus alepidatus*, harvest fish
24. *Leptocephalus conger*, conger eel
25. *Prionotus carolinus*, brown sea robin
26. *Trichiurus lepturus*, cutlass fish
27. *Anguilla rostrata*, common eel
28. *Paralichthys dentatus*, fluke
29. *Pseudopleuronectes americanus*, flounder
30. *Opsanus tau*, toadfish
31. *Lophopsetta maculata*, sand flounder

Hughes (1966)

32. *Trachurus trachurus*, horse mackerel
33. *Clupea harengus*, Atlantic herring
34. *Gadus merlangus*, whiting
35. *Onos mustela*, five-bearded rockling
36. *Grenilabrus melops*, gilt head
37. *Salmo trutta* sp., brown trout
38. *Tinca tinca*, tench
39. *Chaenocephalus* sp., icefish
40. *Lophius piscatorius*, angler, monkfish
41. *Pleuronectes platessa*, plaice
42. *Zeus faber*, john dory
43. *Trigla gurnardus*, grey gurnard
44. *Cottus bubalis*, sculpin
45. *Callionymus lyra*, dragonet

Saunders (1962)

46. *Cyprinus carpio*, carp
47. *Catostomus commersoni*, white sucker
48. *Ictalurus nebulosus*, brown bullhead

Price (1931)

Micropterus dolomieu, small-mouthed bass

Muir and Hughes (in preparation)

Katsuwonus pelamis, skipjack tuna
Thunnus thynnus, bluefin tuna
Thunnus albacares, yellowfin tuna

So far as they are available, values for the number of secondary lamellae per millimeter of filament are plotted (log–log) against fish weight in Fig. 2. (The values are for one side of the filament only. There is an equal number on the other side). The line for tuna was fitted by regression (slope = –0.11) and the lines for bass, sucker, and bullhead fitted by eye (points not shown). For the other species, for which more than one size was examined, lines joining the data points are variable but the evidence strongly indicates that the number of lamellae per millimeter of filament decreases with fish size and that the coefficient of decrease may be of the order of –0.1 for several species. The consequence is that although a small bass may have the same number of lamellae per milli-

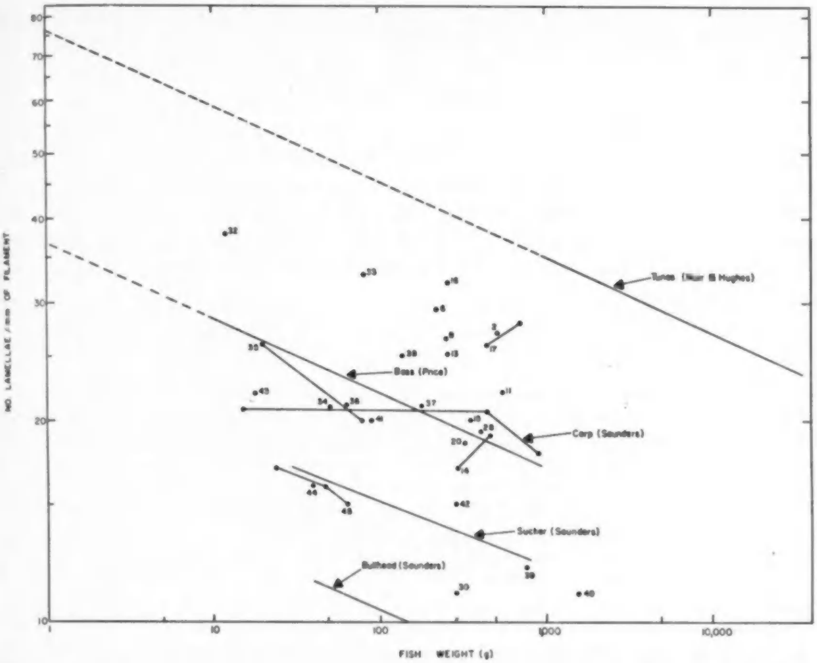

FIG. 2. Relationship between fish weight and the number of lamellae per millimeter of filament for various species of fish. Numbers refer to list in Table 1. See text.

meter of filament as a large tuna, the bass actually has only about half as many as the tuna of the same weight. At 100 g, the number of lamellae range from about 10.5 for the bullhead to about 45 for the tuna. Muir and Hughes (in preparation) found that a skipjack tuna of about 1 g had an average of about 60 lamellae per millimeter which agreed with the regression equation for this species (it is lower than the value given here for three species of tuna combined).

It may be concluded that for at least some species of teleosts the total respiratory surface area increases with weight to a power ranging from about 0.8 to 0.9. This leads to the rejection of the hypothesis that gill area is proportional to weight (i.e. that the power of increase is unity). Similarly, the second hypothesis, that the number of secondary lamellae per millimeter of filament is constant (power of decrease with increasing fish size is zero), is rejected for it is concluded that the number decreases with fish weight to a power of about −0.1.

A decrease in the number of lamellae per millimeter of filament means, of course, that the distance between them — the thickness of the water channel — increases with size of fish. This is particularly interesting for the resistance to flow is a function of both the length and the thickness of the channel. The length of the channel (length of secondary lamellae) increases with fish size and the simultaneous increase in channel thickness would tend to keep the resistance from getting too large. The thickness of the channel determines the length of the diffusion pathway for the dissolved gases, and the length of the channel determines the time during which diffusion can take place for a given water velocity. Evidently, judging from the range in the number of secondary lamellae per millimeter, each species combines the variables in a different way. Whatever the specific combination, however, the pattern of change with increasing fish size may be quite similar for most species. The present examination demonstrates the need to account for the absolute size of the fish in making species comparisons.

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Physiographic Changes in Malpeque Bay, Prince Edward Island, Between 1845 and 1955, and the Possible Effects on Oyster Production[1]

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Recent changes in depth and coastal morphology were investigated by comparing old and new charts. All major changes seem to be the result of normal morphological evolution. Artificial changes, induced by human activity, seem to be relatively slight. It is widely believed that the decline of oyster populations was caused by silt deposition, and this in turn was produced by increased runoff after human occupation (farming, forestry, etc.). Silting has occurred in certain areas, but this was due to natural causes. The decline of oyster populations was therefore due either to natural causes or to factors other than silting.

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Malpeque Bay is on the north coast of Prince Edward Island and is part of a series of lagoons and estuaries which form a more or less continuous physiographic unit. It is shallow: the greatest depth is little more than 14 m, and over most of its area it is fully saline. Several inlets, locally called "rivers," open into the bay and most of these have small streams running into them. Salinity is reduced where these streams enter the inlets, but the effect is small even during the spring run-off because of the very small drainage area of the streams (Needler, 1931). Malpeque Bay is, therefore, not truly estuarine, but the area as a whole can be considered as a lagoonal and estuarine complex. The surrounding area is covered with glacial till derived from underlying friable sandstones and siltstones of Permo–Carboniferous age.

In the latter part of the 19th century, Malpeque Bay produced considerable quantities of oysters, but by 1900 production had declined. This decline continued until 1915, when disease suddenly reduced the stocks to a very low level and no oysters were harvested. By 1935 a disease-resistant stock had developed and harvesting was again possible but only a few thousand barrels per year were taken. This rate of production has not increased very much and it is widely believed that, among other things, the deposition of silt has had a deleterious effect on the oyster stocks and has prevented their rapid recovery.

We have followed morphological changes in the bay by comparing two charts. One was published in 1912 by the United States Hydrographic Office, but was based on a British Admiralty survey of 1845. The other was by the

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[1] Contribution 141 from the Bedford Institute.

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Canadian Hydrographic Service (surveyed 1955–58). As the charts were printed on different scales, we prepared overlays to a common scale of 1:37,500. On comparing these, we found that the latitude lines were coincident but the longitude lines were displaced by about a mile. Apparently the longitude had been re-defined at some time between 1845 and 1955 and the overlays could not therefore be put into east–west coincidence. However, two fixed points common to both charts were found: (1) the church spire at Malpeque and (2) the church spire at Grand River. The overlays could then be fixed in relative coincidence with these points and the latitude lines. Differences between the overlays were then due to morphological changes during the period 1845–1955.

Depth changes were also investigated. Unfortunately no datum was given on the earlier chart, but it has been the experience of the Canadian Hydrographic Service that there is very little difference in datum between early British Admiralty surveys and more recent Canadian surveys. Therefore, in those areas where the depth has changed the differences in depth represent erosion or accretion during the interval between the surveys. It is assumed that no significant subsidence or uplift has taken place.

The most prominent coastal changes have been in the channels at the mouth of the Bay. Originally there were two channels: one (West Gully) was about 230 m wide and 6 m deep and ran northeast; the other (Ship Channel) was about 460 m wide and 15 m deep and ran east-northeast. West Gully was completely filled by 1951 and now only Ship Channel remains. Other coastal changes have been slight and are more apparent at low water mark (Fig. 2) than at high water mark (Fig. 1).

The pattern of erosion and deposition, as inferred from depth changes, is shown in Fig. 3. The main features of this are: (1) considerable deposition in Bideford River (average about 3.5 m) and an area of more moderate deposition extending from there into the Bay; (2) considerable erosion in Ship Channel (average about 6 m) and an area of more moderate erosion extending from there

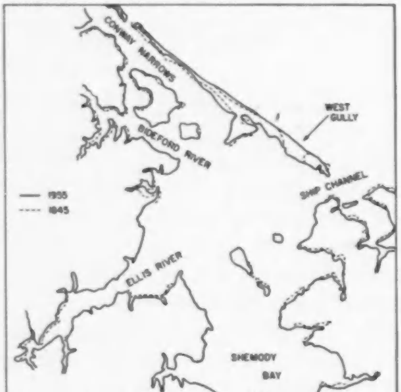

FIG. 1. Coastal changes at high water mark.

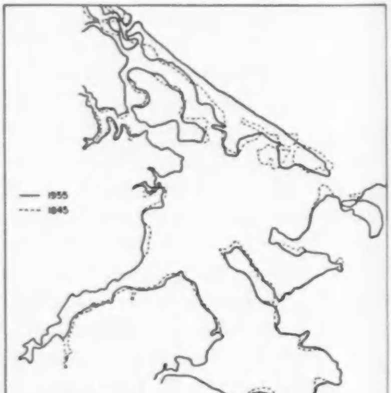

FIG. 2. Coastal changes at low water mark.

into the Bay; (3) moderate deposition (average less than 1 m) in the middle of Shemody Bay. These changes are probably all associated with, or are a result of, the silting of West Gully.

Although there have been erosional and depositional changes, there has been relatively little net gain of sediment. There are considerable areas where the depth of water has not changed and where there has, therefore, been no net gain or loss of sediment. There are other areas where the amount of erosion and deposition very nearly balance. For instance, in Ellis River 4.74 × 10^6 m^3 have been eroded and 4.56 × 10^6 m^3 have been deposited, and similar reworking has taken place around the periphery of Shemody Bay (10.39 × 10^6 m^3 erosion and 11.51 × 10^6 m^3 deposition). The total deposition in Malpeque Bay has been about 82.7 × 10^6 m^3, but at the same time 65.6 × 10^6 m^3 have been eroded. This gives a net gain of only 17.1 × 10^6 m^3 in 110 years. If this were spread evenly over the bay (the total area below water being about 16,800 ha), it would form a layer only 10.7 cm thick. Sedimentation rates in estuarine and lagoonal environments are variable but generally range from 15 to 20 cm per century (Rusnak, 1967). On this basis the average rate of sedimentation in Malpeque Bay is relatively slow. The morphological changes which have taken place are a normal feature of the slow geological evolution of a lagoonal and estuarine complex. From a geomorphic viewpoint the Malpeque Bay of the mid 20th century is not significantly different from that of the mid 19th century.

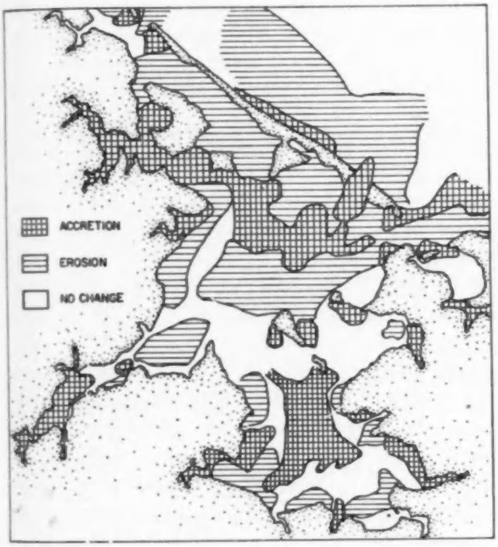

FIG. 3. Pattern of net erosion and accretion between 1845 and 1955.

Therefore, in the bay as a whole the recovery of oyster stocks has probably not been inhibited by net deposition of sediment. However, sediment deposition is only one of the many environmental factors which could affect the stability of oyster populations. Our simple comparison of present and past topography gives only *net* deposition or erosion and gives no indication of the intensity of short-term sediment reworking. Considerable quantities of sediment can be moved back and forth with the tide and may be deposited and eroded many times (Kestner, 1961), and the amount of sediment in suspension may be hundreds of times greater than the amount which is deposited (Postma, 1961; Sheldon, 1968). However, these conditions are typical of lagoonal and estuarine environments, and as oysters are also typical of these environments it is reasonable to assume that normal intensities of short-term sediment reworking and normal concentrations of sediment in suspension should have no significant long-term effect on oyster populations.

But what is "normal"? Conditions in some areas of the bay are probably abnormal. For instance it is common knowledge that oyster beds in Bideford River have been covered with excessive amounts of silt. This is probably because the sedimentary environment has changed relatively rapidly in response to the changed circulation pattern caused by the silting of West Gully. The Bideford River area has not yet reached equilibrium, for not only has sediment accumulated to a depth of more than 3.5 m since 1845, but the sedimentary environment is still changing rapidly.

Although there is very little published information, it has generally been assumed both by scientists and by others concerned with the oyster industry that much silt has been washed from the land and deposited on oyster beds as a result of forest clearing, farming, and road building. This is unlikely. Some land-derived material has been deposited in the heads of the creeks but most of the sedimentary deposition is due to reworking of material already in the bay.

Malpeque Bay is following a normal course of geological evolution, and a recent, rapid, overall change in sedimentary environment is unlikely. Although it is possible that recovery of oyster stocks could be inhibited by changes in the pattern of sedimentation, it is more probable that heavy silting of oyster beds has occurred only in relatively localised areas where the sedimentation rate is temporarily high. In the light of this we consider that the recommendation of Needler (1931, 1941), that oyster beds should be laid in the inlets, should be re-examined, and attention should be directed to the discovery by C. S. Sharp (cited by Medcof, 1961) that spat can be reared on the bottom in Conway Narrows, an area of gentle erosion. From the geomorphic evidence alone it seems that areas which were once suitable for oysters are now unsuitable, and it is possible that formerly unproductive areas are now suitable for oyster populations.

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Identification of Northern Pike and Muskellunge from Axial Skeletons, Scales, and Epipleurals

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Grooves and knobs on the vertebrae, ratio of length and width of scales, and ratio of lengths of rami of epipleural bones enabled us to separate northern pike and muskellunge in mutilated specimens, stomach contents, and archaeological material.

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Often species of fishes in the same genus are assumed to share osteological characters considered to be at the generic level. Detailed examination of many of these reveals that they are specifically distinct and can be used to differentiate between members of the genus.

When fish are caught out of season or by illegal means, the offender attempts to prepare the flesh in a way which he feels renders it unidentifiable to a law enforcement officer, or at least indistinguishable from another, legal species. Such methods include various combinations of evisceration: removal of head, all fins, and scales; skinning; and steaking or filleting. Fish remains from archeological sites often consist of vertebral centra but none of the more fragile and more readily identifiable skull bones. The identification to species of fishes in the stomachs of other fishes frequently depends solely on the availability of distinguishing characteristics of bones.

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Various parts of fishes from sources mentioned above, submitted for identification, led to the reexamination of many characters which might differentiate between northern pike, *Esox lucius* Linnaeus, and muskellunge, *Esox masquinongy* Mitchill. The two most recent examples consisted of several pounds of fillets without skin, and of a single large steak from the midportion of a fish from which scales and colour pattern had been removed. Shape, texture, odour, colour, and other superficial characters readily identified the material as belonging to a member of the pike family (Esocidae). In each case careful examination of the surface revealed that scales in the midventral area had been missed, distinctive Y-shaped epipleural or intramuscular bones were present in both pieces, and about 4 inches of the vertebral column was intact in the steak. On the basis of these three items, positive identifications were made. These characters are outlined below both as a contribution to our knowledge of osteological variability and as an aid in the identification of stomach contents, archeological material, and in law enforcement problems.

Scale size and shape— The cycloid esocid scales key out readily in the key published by Lagler (1947). Scale shape varies from one part of the body to another but ratios of the maximum scale length to the maximum scale width separate the two species (Williams, MS, 1955). The range of the ratios for 10 scales from each of two fish was 1.77–1.98 for *E. lucius* (700–900 mm) and 1.41–1.49 for *E. masquinongy* (653–875 mm). Ratios for scales from the midventral region found on the unidentified steak ranged from 1.30 to 1.58. These values exceed those of known *E. masquinongy* but did not overlap values for *E. lucius*. Scales of *E. masquinongy* appear wide for their length, or circular (Fig. 1A) whereas scales of *E. lucius* appear oval or long and narrow (Fig. 1B). This character seems useable for scales from any area of the body.

J. E. Williams (personal communication) noted the distinction between these two species in the number of lobes and extent of overlap of the lobes of the anterior field (imbedded portion) of the scale. Scales of known *E. lucius* taken from the same area as those found on the unidentified material are rarely (less than 1 in 20) simple and complete in the outline of the anterior field. There are usually three, rarely two, and sometimes four, lobes to the anterior margin. These overlap one another widely at the tips, and the overlap continues down the radii almost to the focus. Scales of muskellunge from the area usually missed in scaling, i.e. the midventral region, are more often complete (15 out of 20) and when lobed have never more than three and often (8 out of 20) only two lobes. If the scales are lobed, the lobes overlap not at all, or to a limited degree at the tips. This overlap does not continue for more than one-third the length of the radii. However, scales from the midlateral area, from which scale samples are usually taken, often do not exhibit this difference. Scales in Fig. 1A and B are from the midlateral region. Therefore a combination of the ratio of scale length to scale width and the nature of the lobing of scales will usually separate the two species. Scales provide the least reliable characteristics. It would require the study of many more scales to substantiate them as a single distinguishing feature. They can, however, help corroborate other findings.

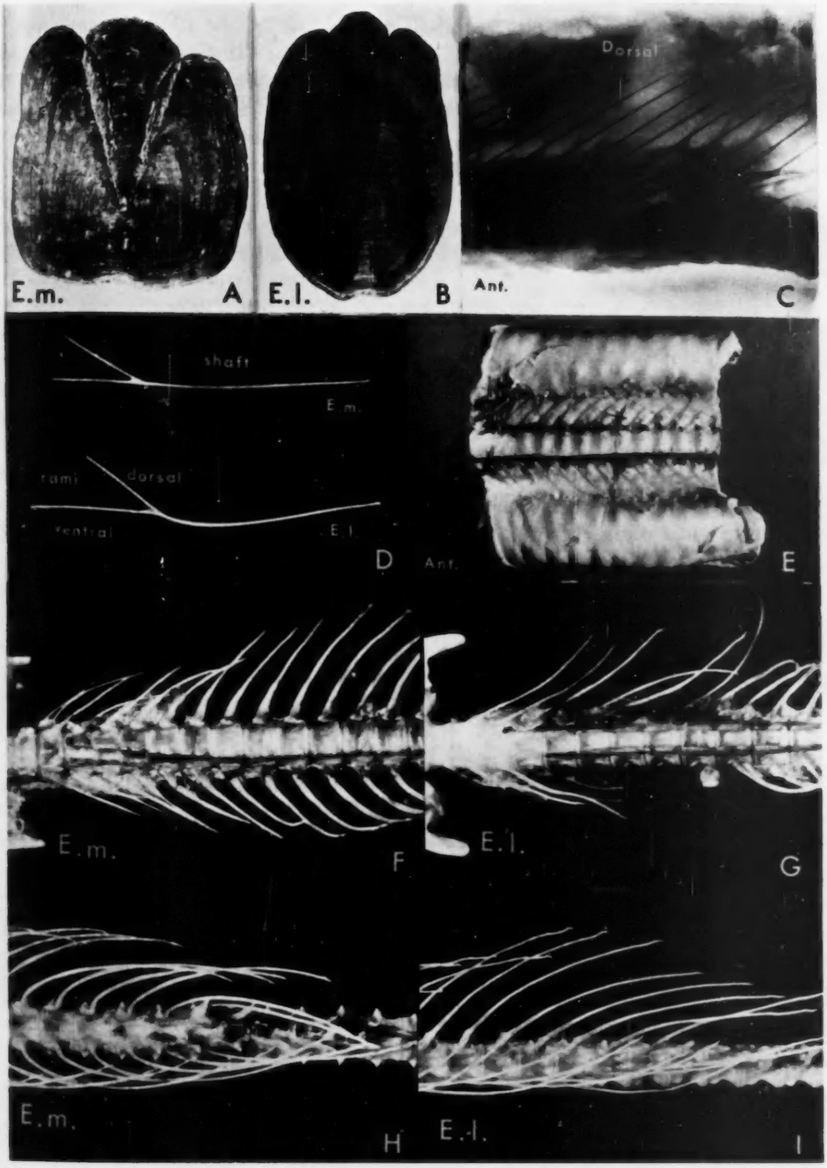

FIG. 1. A, Scale from mid-lateral region of *Esox masquinongy*. B, Scale from midlateral region of *Esox lucius*. C, Radiograph of the epaxial muscle mass, removed as a fillet, from the left side of *E. masquinongy*. D, Epipleurals from *E. masquinongy* (*above*) and *E. lucius* (*below*). E, Ventral view of a steak from the region just anterior to the pelvic fins of *E. masquinongy*. The dark line at the right side of the vertebral column is the dorsal aorta. F, Ventral view of the anterior portion of the axial skeleton of *E. masquinongy* showing curved path of groove for dorsal aorta. G, Ventral view of the anterior portion of the axial skeleton of *E. lucius* showing straight path of groove for dorsal aorta. H, Ventral view of posterior portion of axial skeleton of *E. masquinongy* showing conspicuous lateral spines. I, Ventral view of posterior portion of axial skeleton of *E. lucius* showing short, inconspicuous lateral spines.

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Epipleurals— In filleted esocids the Y-shaped epipleurals or intramuscular bones show very distinctly in a radiograph (Fig. 1C). The epipleurals of the two species are superficially similar but relative lengths of the shaft, dorsal ramus, and ventral ramus differ (Fig. 1D). The only epipleural characteristic by which the two can be distinguished, over all sizes, is the ratio of the lengths of the two rami. A series of epipleurals from one side of any individual exhibits a considerable range in the length of individual epipleurals and rami. Values for length of dorsal ramus ÷ length of ventral ramus for all of the Y-shaped epipleurals from single sides of five pike and three muskellunge between 487 and 1326 mm total length are given in Table 1.

Both the minimum and the maximum ratios are greater in muskellunge than in the pike, but there is considerable overlap in the range of values. The mean ratios for series of epipleurals show no overlap in the two species. No single epipleural can be trusted to distinguish the two but a series can. Any fillet contains an adequate series. The overlap in ratios is the result of changes in the size of component parts of epipleurals in the area where the muscles taper from the straight line of the back to the caudal peduncle. The largest epipleurals occur in the region just anterior to the pelvic fins. If these are used for comparison there is no overlap, separation of species is complete, possibly even on single bones. In this region the DR/VR ratio is $1<$ for *E. lucius* and >1 for *E. masquinongy*.

Comparing individual fish of the same length, and epipleurals from the same point in the series, those of *E. lucius* tend to be finer, smaller, and more curved than those in *E. masquinongy*. However, for Fig. 1D, epipleurals of similar length were chosen. The size difference seems reversed in the figure,

TABLE 1. Values for the epipleural ratio $\frac{\text{dorsal ramus length}}{\text{ventral ramus length}}$ for complete series of forked epipleurals from one side of individual *Esox lucius* and *E. masquinongy*.

E. lucius			*E. masquinongy*		
T.L.[a] (*mm*)	Range of ratios for individual epipleurals	$\overline{X}$	T.L.[a] (*mm*)	Range of ratios for individual epipleurals	$\overline{X}$
595	0.50–1.10	0.77	487	0.81–1.75	1.43
608	0.34–1.41	0.86	487	0.71–1.71	1.42
608	0.39–1.55	0.88	489	0.73–1.93	1.39
627	0.32–1.51	0.78	489	0.77–1.63	1.17
627	0.59–1.57	0.85	1326	0.78–1.85	1.85
631	0.44–1.19	0.88			
631	0.50–1.39	0.86			
679	0.56–1.48	0.87			
679	0.52–1.45	0.84			

[a] T.L. = total length.

but the upper bone is from a muskellunge 489 mm long and the lower from a northern pike 627 mm long. The other distinctive feature, apparent in the figure, is the greater curvature of the shaft in *E. lucius*.

Vertebral column— In other studies of the bones of the members of the family Esocidae an interesting anomaly became apparent. The dorsal aorta runs in a shallow groove on the ventral surface of the vertebral centra. In all species except *E. masquinongy* this groove is midventral over the whole length of the vertebral column (Fig. 1G). In *E. masquinongy* the dorsal aorta runs in a central groove for the anterior three to six vertebrae, shifts gradually, over the next three to five vertebrae, to a more dorso-lateral position adjacent to the bases of the ribs of the right side (Fig. 1E and F). The aorta continues in this position to the 26–28 vertebra, where it shifts again to a midventral position and eventually passes inside the haemal arches. This difference was constant in a large series of specimens and skeletons examined. In hybrids between *E. masquinongy* and other members of the family, the dorsal aorta is in the *lucius* or midventral position. On the basis of this difference in the position of the aorta, it is simple to identify fresh material from the region of the anterior ribs, if the vertebral column is intact (Fig. 1E).

Beyond the point where the groove in the muskellunge returns to a position similar to that of the northern pike, still another feature differentiates them. On the mid-lateral aspect of about the last five abdominal centra and all the caudal centra, there are short, sharp spines. In *E. masquinongy* these project noticeably beyond the maximum diameter of the centra, whereas in *E. lucius* they are inconspicuous and do not protrude beyond the ridges of the centra (Fig. 1H and I).

There are also differences in the sculpturing of individual vertebra. In *E. lucius* each vertebra, after the fourth or fifth, to the end of the ribs has a deep, smooth midventral depression bordered on all sides by ridges. In fresh material the overlying fascia may have to be removed to show these distinctly. They are very obvious in prepared skeletons. The rib-bearing vertebrae in *E. masquinongy* lack these obvious midventral pits and instead have smooth to rough, uniform surfaces (Fig. 1F and G).

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An Application of Computers to the Estimation of Exploited Populations

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Allen, K. Radway. 1969. *An application of computers to the estimation of exploited populations.* J. Fish. Res. Bd. Canada 26: 179–189.

This paper describes a computer programme for the estimation of the size of exploited populations by methods described in Allen (1966, J. Fish. Res. Bd. Canada 23: 1553–1574). Although these methods were originally developed for use on whale populations, they are applicable to any other populations where suitable data are available. The essential data are the total catch, the catch for a known amount of effort, and the age distribution of the catch, all for a series of years. An estimate of natural mortality rate is also required but population estimates may be obtained for up to 10 values of this parameter in a single computer run.

The entire programme incorporates six subroutines, as well as the main controlling programme. One subroutine reads in the catch and effort data, a second reads in the age distribution data for each year and, if necessary, converts it according to a predetermined transformation from age expressed in terms of whale ear-plug laminations to age in years. The third subroutine estimates the rate of recruitment as the proportion of newly recruited animals in the catch for each year, using the method of Allen (1966). The other three subroutines derive population estimates, using the "*q*," modified DeLury, and actual and expected catch methods (Allen, 1966). As many sets of data as desired may be processed in a single computer run.

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Application of some of the more complex methods of estimating populations may sometimes be limited by the extensive and laborious calculations involved. The magnitude of the calculations may be due both to the nature of the mathematical techniques and to the large amount of data which must be handled, particularly in methods which involve the examination of prolonged time series. The development of modern computers has, however, made practicable many analyses, in this as in other fields, which would previously have been impossible or economically impracticable.

This paper describes a computer programme which has been constructed to carry out a series of population estimates by a set of methods described in Allen (1966b). Although these methods were originally developed in connection with studies on the whale populations of the North Pacific Ocean, they are also suitable for the estimation of other populations for which data are available for a series of years on the total catch, the catch for a known amount of effort, and the age distribution of the catch. They seem therefore to be potentially applicable to many commercially exploited fish populations. In addition to the rapid performance of the complex calculations on large quantities of data

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which these methods involve, the programme utilizes the capacity of the computer to hold the basic data in storage and to carry out as many operations as desired upon it. It also tests, where desired, the effect on any of the estimates of trying a series of different values for any parameters which cannot be directly estimated.

As is usual in computer programmes of any complexity, and particularly in those in which major sections may have to be repeated several times, the programme is broken up into a series of routines, each of which performs a major segment of the calculations. In the present programme there are seven such routines, each of which is identified by a more-or-less appropriate code name. These consist of one principal routine, MAIN, which reads in the values of the various fixed parameters, and controls the order of functioning of the other parts of the programme and the flow of data between them; two subroutines, CEFF and YEARX, which read in the basic data of catch and effort, and age distribution, respectively, and convert them into forms which can be used by the other subroutines; one subroutine, RECRUT, which estimates recruitment rates from the age distributions; and three subroutines, QUE, POPREC, and LSTSQ, which compute population estimates by the three methods described in Allen (1966b).

Details of the methods of calculation, together with numerical examples extracted from computer output, are given in Allen (1966b).

Operation of subroutines— The following operations are performed by the six subroutines.

(*1*) *Catch and effort subroutine* (CEFF)— This reads in data for each year covering the known amount of effort and, for each sex, the total number of animals caught, and the number caught for which the effort was known. It also reads in an effort modifier, variable from year to year, which permits adjustment of the recorded effort for other measurable effects (e.g., weather). It determines, for use in later stages of the programme, the catch per unit modified effort, and the total effort, on the assumption that the catch per unit effort was the same for the entire catch as for that part for which the effort was known.

(*2*) *Age-converting subroutine* (YEARX)— This converts an age distribution based on units of time which can be directly counted into an age distribution based on years. It is the only part of the programme specifically designed to meet the special peculiarities of whale data. For determining the age of baleen whales, the method most widely used at present is to count the number of laminations in the ear plugs. Probably either one or two of these laminations are formed annually, and it is possible that two are formed each year in young animals and one in older animals. The programme converts age in laminations to age in years by counting laminations in twos up to a predetermined age and singly thereafter. The age of change from two to one lamination annually is read in through the MAIN routine. By setting this age at 0 or at the maximum permitted age (42 years), conversion entirely at one-for-one, or two-for-one may be obtained. The former would be appropriate for handling fish data in general. The input data are in the form of a cumulative distribution of actual numbers, summed from the higher end, and are designed to be read from the output cards of another programme (Allen, 1966a), which obtains an age distribution from a length distribution and an age–length key by standard methods. The output is a simple proportional distribution of age in years.

(*3*) ***Population subroutine*** (QUE)— This uses the "*q*" method of estimating populations described in Allen (1966b). Basically, the method estimates the total instantaneous rate of fishing mortality over a period (ΣF_t) as the natural logarithm of the ratio between the abundances of the same group of year-classes in the first and last years of the period; a correction for natural mortality is made by dividing this ratio by the ratio between the abundances of the corresponding age-groups in a single year which is selected as having an age distribution unaffected by exploitation.

The basic equation used in the programme is

$$\Sigma F_t = ln\left[\frac{Q_{1,r}}{Q_{t,r+t-1}} \cdot \frac{Q_{0,r+t-1}}{Q_{0,r}} \cdot \frac{\varphi_1}{\varphi_t}\right]$$

where ΣF_t = total instantaneous fishing mortality from year 1 to year t; $Q_{1,r}$ = proportion in the catch in year 1 of whales of age r (the first fully recruited age) and older; $Q_{t,r+t-1}$ = proportion in the catch in year t of the same age-groups; $Q_{0,r}$ = proportion in the catch from the previously unexploited stock of whales of age r and older; $Q_{0,r+t-1}$ = proportion in the same year of whales age $r+t-1$ and older; $\varnothing_1$ = catch per unit effort in year 1; $\varnothing_t$ = catch per unit effort in year t.

Dividing ΣF_t by the corresponding effort gives an estimate of the catchability coefficient q. In the programme, as in Allen (1966b), a weighted mean value of q is obtained. For this purpose the values of ΣF_t are summed for all the periods beginning with the first year of observations and ending with each of the subsequent years; this total is divided by a similar compound sum of efforts.

An estimate of the mean population in each year is then obtained by dividing the catch per unit effort in that year by the mean q.

The information required by this subroutine is therefore (*a*) the age distribution in each year for which the population is to be estimated, and for a year when it had not been affected by exploitation; (*b*) the catch per unit effort in each year for which the population is to be estimated; (*c*) definition of the period for which population estimates are required; (*d*) definition of a year when the age distribution was unaffected by exploitation. Of these, (*a*) is supplied by subroutine YEARX, (*b*) by subroutine CEFF, and (*c*) and (*d*) by MAIN. This subroutine also obtains annual estimates of the instantaneous fishing mortality rate (F) as the product of the annual effort and mean q.

(4) *Recruitment subroutine* (RECRUT)— This obtains from the age distribution data an estimate of the proportion of new recruits in the catch in each year using the method described by Allen (1966b). The programme constructs a succession of two-dimensional matrices with years as columns, and ages up to and including the first fully recruited age-group as rows. The initial matrix is composed of the proportions of the age-group in the catches of each year ($P_{a,t}$). From this a matrix is computed of the quantity B where

$$B_{a,t} = \frac{P_{a+1,t+1} \cdot Q_{r,t}}{P_{a,t} \cdot Q_{r+1,t+1}}$$

where $Q_{r,t}$ is the cumulative proportion of all age-groups from the first fully recruited age-group and up, in year t. A matrix of the quantity U is computed from this where $U_{a,t}$ is the proportion of the a^{th} age-group which is in the recruited part of the population in year t and

$$U_{a,t} = \frac{e^{F_t}}{\dfrac{B}{TU_{a+1,t+1}} - 1 + e^{F_t}}.$$

Since the value of $U_{a,t}$ depends on $U_{a+1,t+1}$, the matrix is constructed by starting in the last column and the lowest row but one, taking advantage of the fact that $U = 1$ for the whole of the bottom row, and assuming an additional column at the right of the matrix and identical with the real last column.

In this calculation F_t is the instantaneous fishing mortality rate in year t, and T is the ratio between the survival rate of the recruited part of the younger year-classes and that of the older animals. The third matrix computed is that of $R_{a,t}$, the proportion of new recruits in the recruited part of the a^{th} age-group in year t. This is computed from

$$R_{a,t} = 1 - \frac{U_{a-1,t-1}}{U_{a,t}} \cdot \frac{1}{U_{a-1,t-1} + (1-U_{a-1,t-1})\, e^{F_{t-1}}}.$$

Finally, the proportion of new recruits in the catch of each year, W_t, is computed as the sum of the products, for each age-group, of its proportion in the catch and the proportion of new recruits in the recruited part of it.

This subroutine therefore requires (*a*) the age distribution for each year; (*b*) the fishing mortality rate in each year; (*c*) an estimate of T.

As before (*a*) is supplied from YEARX, (*b*) can be obtained from QUE, and for (*c*) the value of T is read in through MAIN, being usually taken to be 1.0.

(5) *Population subroutine* (POPREC)— This uses the modified DeLury method, making allowance for natural mortality and recruitment, described in Allen (1966b). The basic equation for this method is

$$N_1 = \frac{\sum_{1}^{t-1} C_i}{1 - Y_t - M\sum_{1}^{t-1} Y_i + \sum_{2}^{t} W_i Y_i}$$

where N_1 = population at the midpoint of the first year of a series; Y_i = catch per unit effort in year i/catch per unit effort in year 1; and C_i = mean catch in years i and $i+1$; other symbols are as before.

In the programme, each year in the series being studied is taken in turn as year 1 and a set of estimates is obtained for the population in that year by taking each of the available later years as year t. The mean of each set is taken as the estimated population for that year.

The information required by this subroutine is therefore (*a*) the catch in each year; (*b*) the catch per unit effort in each year; (*c*) the proportion of new recruits in the catch in each year; (*d*) the natural mortality rate. Of these, (*a*) and (*b*) are obtained from subroutine CEFF, (*c*) from subroutine RECRUT, and (*d*) is read in through MAIN.

(*6*) ***Population subroutine*** (LSTSQ)— This subroutine obtains population estimates by a method which minimizes the sums of squares of the differences between actual and expected catches (Allen, 1966b). The estimated population for the beginning of the base year is given by

$$N_1 = \frac{LR - 2PU}{RP - 2SL}$$

where $L = \Sigma C_t^2 X_t - 2\,\Sigma C_t X_t A_t G_{t-1}$

$P = -2\,\Sigma C_t X_t A_t$

$R = -\,[\Sigma C_t X_t^2 A_t + 2\Sigma X_t^2 A_t^2 G_{t-1}]$

$S = \Sigma X_t^2\, A_t^2$

$U = 1/4\Sigma C_t^2 X_t^2 + \Sigma C_t X_t^2 A_t G_{t-1} + \Sigma X_t^2 A_t^2 G_{t-1}^2$

in which $A_t = \dfrac{e^{-(t-1)M}}{\prod_{i=2}^{t}(1-W_i)}$

$$G_{t-1} = C_1 + \sum_{i=2}^{t-1} \frac{C_i}{A_i}$$

and X_t = effort in year t.

The population in subsequent years is given by

TABLE 1. Summary of input, generated functions, and output of each subroutine.

Routine	Reads	Receives	Generates	To other routines	Output
MAIN	Age of change (from 2 laminations/year to 1 lamination/year) Natural mortality rate Range of years (for estimates) Age at full recruitment q	Fishing mortality rate^{a,b} Modified effort^{b}	Fishing mortality rate^{a,b}	Age of change Natural mortality rate Fishing mortality rate^{a,b} Range of years Age at full recruitment	
CEFF	Catch^{b} (by sex) Catch for known effort^{b} (by sex) Known effort^{b} Effort modifier^{b}		Total modified effort^{b} Total catch^{b} Catch per unit modified effort^{b} (by sex and total)	Total modified effort^{b} Total catch^{b}	Input data Total effort^{b} Total modified effort^{b} Catch per effort^{b} (by sex and total)
YEARX	Cumulative lamination distribution^{b} (by sex)	Age of change	Proportional age distribution^{b} (by sex) Cumulative age distribution^{b} (by sex)	Proportional age distribution^{b} (by sex)	Proportional age distribution^{b} (by sex) Cumulative age distribution^{b} (by sex)
QUE		Range of years Proportional age distribution^{b} Catch per effort^{b} (by sex and total) Total modified effort^{b} Age at full recruitment	Fishing mortality rate^{a,b} Population^{b} (by sex, total and combined) q	Fishing mortality rate^{a,b}	Population^{b} (by sex, total and combined) q

RECRUT	Range of years Fishing mortality rate[b] Proportional age distribution[b] Age at full recruitment	Proportion of new recruits in catch[b] (by sex)	Proportion of new recruits[b] (by sex)	Proportion of new recruits[b] (by sex)
POPREC	Total catch[b] Proportion of new recruits[b] Range of years Natural mortality rate Catch per effort (total)	Population (total)[b] q^b		Population (total)[b] q^b
LSTSQ	Total catch[b] Proportion of new recruits[b] Range of years Natural mortality rate Total modified effort[b]	Population (total)[b] Fishing mortality rate[a,b] q	Fishing mortality rate[a,b]	Population (total)[b] q

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[a] Alternative values of fishing mortality rate are produced by different paths.
[b] Annual values are read, computed, or printed.

$$N_t = A_t \left[N_1 - G_{t-1}\right]$$

The subroutine also obtains an estimate of q, using

$$q = \frac{RP-2SL}{4SU-R^2}$$

and annual estimates of F as qX_t.

The information required by this subroutine is (*a*) the catch in each year; (*b*) the effort in each year; (*c*) the proportion of new recruits in the catch in each year; (*d*) the natural mortality rate. Of these, (*a*) and (*b*) are supplied by subroutine CEFF, (*c*) is obtained from subroutine RECRUT, and (*d*) is read in by MAIN.

Organization— When the subroutines are listed in the above order, the information required by each of them is available either from MAIN or from a preceding subroutine. This order is therefore the basic order of operation of the programme.

The functions and input and output of each section of the programme are summarized in Table 1.

Considering the programme as a whole the information needed consists of (*a*) the catch and effort data required by CEFF; (*b*) the annual age distributions used by YEARX; (*c*) the following control values and parameters read in through MAIN:

(*i*) The age at which laminations change from 2 to 1 per year in YEARX.

(*ii*) The range of years over which the population and recruitment estimates will be computed. This may be selected as any part of the full range of data processed by CEFF and YEARX. This allows any period at either end of the full range to be omitted if desired, if, for example, the data are unsatisfactory or inconsistent.

(*iii*) Age at full recruitment.

(*iv*) The year for which the age distribution is assumed to have been developed in the absence of exploitation.

(*v*) The parameter T in the computation of recruitment.

(*vi*) The natural mortality rate.

The natural mortality rate is often difficult to estimate. Provision has therefore been made for up to 10 values to be fed in and population estimates from subroutine POPREC and LSTSQ are obtained for each value in turn. The estimate from subroutine QUE is independent of M.

The fishing mortality rate (F) varies, of course, from year to year. It is initially computed for each year in subroutine QUE, and then fed into RECRUT to give the recruitment values for POPREC and LSTSQ. As, in some circumstances, QUE does not produce very satisfactory estimates, an iteration is provided. After these steps have been completed for each value of M, the new values of

F obtained from LSTSQ are fed back to RECRUT and finally POPREC and LSTSQ are repeated with the new recruitment values. As the recruitment estimates are not very sensitive to the value of F it has not been considered necessary to carry out further iterations.[1]

In old-established fisheries it may not be possible to obtain an age distribution which is unaffected by exploitation. In these circumstances an alternative (Option 2) is used; this omits subroutine QUE entirely, but uses a supplied value of q to obtain preliminary estimates of recruitment, which are then adjusted for each value of M as before, by using values of F obtained from LSTSQ.

Flow-paths— The principal paths followed by input and output data, and between the subroutines, are shown diagrammatically in Fig. 1. The double line shows the path of the various control parameters from the main to the subroutines, and the single lines the paths of the various categories of data and computed results. The broken lines indicate the paths omitted in option 2.

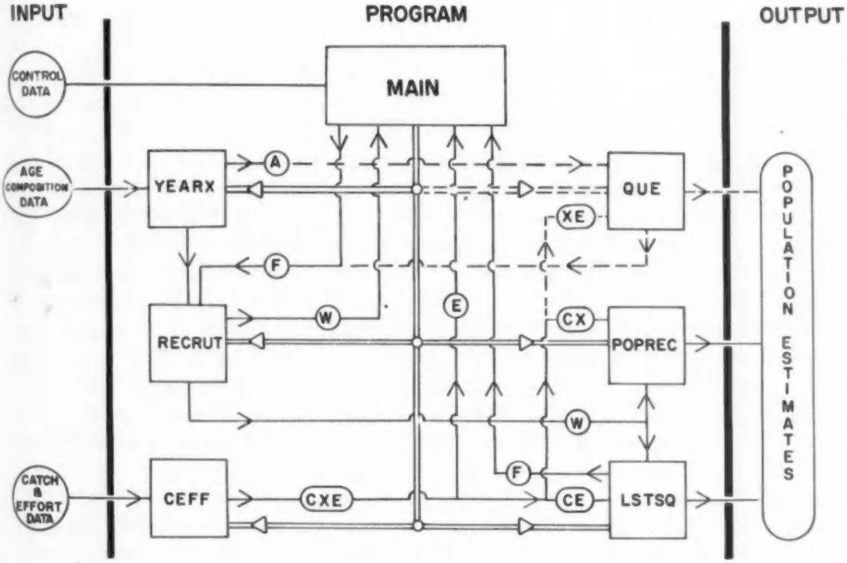

FIG. 1. Principal flow-paths of the programme.

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[1] It has now been found (Allen, 1968) that whereas U is, as stated, slightly sensitive to F, R is independent of it. Since R is the quantity used in subsequent calculations this iteration is unnecessary. Also, a substantial simplification of the RECRUT subroutine is possible. At present, this has only been done for the 1130 Fortran version of this programme.

Output— The printed output consists successively of: (*a*) the summary by years of catch, effort, and catch per unit effort, by sex and total (CEFF); (*b*) the proportional and cumulative age distributions in years for each sex and each year (YEARX); (*c*) the population estimates (by sex and combined) and the estimates of F, from QUE for each year (option 1 only); (*d*) the proportion of new recruits for each sex in each year, using values of F derived from QUE (option 1), or from supplied *q* (option 2) (RECRUT); (*e*) population estimates from POPREC and LSTSQ using recruitment from (*d*); (*f*) recruitment using values of F obtained from LSTSQ; (*g*) population estimates from POPREC and LSTSQ using recruitment from (F); (*h*) items (*e*) to (*g*) repeated for each value of M.

The output for each subroutine on each occasion is labelled with its nature and the source of the data. All outputs include the values of a number of intermediate quantities which may be of assistance in examining the nature of the results.

This programme was originally written in Fortran IV for use with an IBM 7090 computer, and the above description applies to the programme finally developed in this language. More recently it has been necessary to adapt the technique for use on an IBM 1130 computer. The characteristics of this computer, together with increased experience in using these methods of estimating populations, have led to certain changes in the structure of the programme. The principal changes are the elimination of the *q* and modified DeLury methods (subroutines QUE and POPREC), and the dividing of the programme into two parts. As a result of the first change this version of the programme only obtains population estimates by the least square method (subroutine LSTSQ). This has been found to be consistently the most satisfactory of the three methods included in the original programme. Since it has now been found that it is not necessary to use a supplied value of F in estimating recruitment (Allen, 1968), elimination of QUE has not required the supply instead of preliminary values of *q* or F. In the division of the programme the first part terminates with RECRT (equivalent to RECRUT in original version) and gives an output of annual estimates of recruitment rates punched on cards. These become part of the input of the second part which includes LSTSQ and gives a printed output of population estimates as in the original programme.

Minor differences will be apparent in the following technical data regarding the two programmes.

TECHNICAL DATA

A. FORTRAN IV VERSION

Language: The programme is written in Fortran IV and has been run on IBM 7090 and 7094 computers.

Precision: Floating-point single-precision arithmetic is used throughout.

Speed: A set of data covering 4 years and with six values of M requires about 1 min for execution on IBM 7094, and 5 min on IBM 7090.

Storage: About 33,000 positions of core storage are required on IBM 7090.

Continuity: As many sets of data, each with appropriate control cards, may be fed in as desired, and will be processed successively.

Restrictions:

(*a*) The data must lie within a 30-year period. As the programme is now written this must be 1940–69. It would, however, be a simple matter to modify the programme to handle a different period, or, alternatively, data outside this time could be coded by adding or subtracting a constant to make it acceptable provided it did not cover a period longer than 30 years.

(*b*) There is provision for 84 age-groups in the input, although the number of year-groups will be less than this if a conversion other than one-for-one is used.

(*c*) The total number in the age distribution for any sex and year must not exceed 5 significant figures, and must be coded to be less than 1000.

(*d*) The number of animals of either sex caught, or the amount of effort, in any year must be less than 10^9.

B. FORTRAN 1130 VERSION

Language: The programme is written in Fortran 1130 (a subset of Fortran IV) and will run on an IBM 1130 8K under Version 1 Level 7 of Disk Monitor.

Precision: Floating-point single-precision arithmetic is used in the first part of the programme, and extended precision arithmetic in the second part. This limits the number of significant digits to 6 and 10 respectively.

Speed: A set of data covering 10 years and about 30 age-groups requires about 15 min for the first part of the programme, and an initial time of about 1 min 40 sec, followed by 40 sec for the population estimate, and, under an option, a further 4 min for analysis by age-groups, for each value of M in the second part.

Storage: Each part of the programme has been split into several main lines, using the CALL LINK feature, to enable it to be accommodated within the available storage.

Continuity: As for the original version.

Restrictions:

(*a*) The data must lie within any 14-year period. The restriction to a specific range of years in the original programme has been removed.

(*b*), (*c*), (*d*) As for the original version.

Availability— Copies of a source deck for either version of this programme and more detailed instructions can be obtained free on request from the author at Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.

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The Parasitic Copepod *Mytilicola orientalis* in British Columbia Bivalves

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BERNARD, F. R. 1969. *The parasitic copepod Mytilicola orientalis in British Columbia bivalves.* J. Fish. Res. Bd. Canada 26: 190–191.

Under western Canadian conditions infestation of *Mytilicola orientalis* has little economic significance in commercial oyster production. First record of natural infestation of *Saxidomus giganteus* is given.

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THE JAPANESE COPEPOD *Mytilicola orientalis* Mori is found in the recurrent intestine and rectum of various bivalves. This note summarizes a study of the distribution and effect of the parasite in the Pacific oyster *Crassostrea gigas* (Thunberg) and the bay mussel *Mytilus edulis* Linné for the period 1963–67. Full data will be found in Bernard (MS, 1968).

There is divergence of opinion on the significance of *Mytilicola* infestation of commercial shellfish stocks. Koringa (1950, 1952) attributed heavy losses of Dutch *M. edulis* to *Mytilicola intestinalis*. Odlaug (1946) showed that infestation by *M. orientalis* lowered the Condition Factor (CF) of *Ostrea lurida*. It has not been possible to substantiate that presence of the copepod is invariably associated with a depressed condition in the Pacific oyster, for calculations of the CF of 92 infested and copepod-free oysters from Ladysmith Harbour failed to reveal any significant variation between groups.

Sparks (1962) demonstrated that certain metaplastic changes occurred in the gut epithelia of infested oysters, together with a tendency to fibrosis of the underlying tissues. No histological evidence of gross damage has been found in British Columbian Pacific oysters. However, in oysters that have been held out of water for 12 hr or more, there is a tendency to evacuate contained copepods; under these conditions it is possible that the thoracic protuberances of *Mytilicola* will cause lesions on the gut wall.

Distribution is sporadic and limited to the immediate vicinity where infested oyster seed from Japan, or infested adult stock, has been introduced. Nearby areas are free of *Mytilicola*, even where the oyster population is contiguous due to natural spawning.

Under British Columbia conditions there is a single extended reproductive period extending from June to late August, the situation being similar to that reported by Meyer and Mann (1951) for *M. intestinalis* in Germany. Experiments in Ladysmith Harbor indicate that the larval stages are short and do not travel far. Oysters and mussels raised above the substrate are not infested.

The most frequently infested host is *M. edulis*, followed by *C. gigas* and *O. lurida*. Less often *M. californianus* (Chew et al., 1964) and the butter clam *Saxidomus giganteus* may be host to the copepod.

Up to the present there are no indications that *M. orientalis* is of any economic significance in the British Columbia shellfish industry.

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Sinistrality in *Platichthys stellatus* off British Columbia

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FORRESTER, C. R. 1969. *Sinistrality in Platichthys stellatus off British Columbia.* J. Fish. Res. Bd. Canada 26: 191–196.

Percentage sinistrality in the starry flounder (*Platichthys stellatus*) off British Columbia was found to increase proceeding from south to north, and on the average (66%) was greater than that found off the western coast of the United States, and close to that found off Alaska.

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FLATFISHES OF the family Pleuronectidae in the North Pacific are normally dextral, i.e., have eyes and coloration on the right side of the fish. Most frequent exceptions to this pattern occur in the starry flounder, *Platichthys stellatus*, and several authors have shown that reversal in this species is widespread (Norman, 1934; Gudger, 1935; Townsend, 1937). Summaries of sampling conducted from Monterey Bay, California, north through the Gulf of Alaska and off Japan showed that incidence of sinistral specimens of *P. stellatus* ranged from about 50% off Oregon to 100%, or close to 100%, off Japan (Hubbs and Kuronuma, 1942; Orcutt, 1950).

Information on sinistrality or "handedness" in flounders from the British Columbia coast has been collected at intervals since 1945. This previously unpublished material shows that the percentage of sinistral specimens off British Columbia (66.4%) lies above that found for samples taken off Oregon and close to that for samples taken off Kodiak Island and the Alaska Peninsula (Table 1).

TABLE 1. Percentage occurrences[a] of sinistral specimens of *Platichthys stellatus* in the North Pacific Ocean.

Locality	No. of specimens	Sinistral (%)
California	509	55.2
California (Orcutt, 1950)	1439	59.5
Oregon outer coast	65	49.2
Columbia River mouth	225	60.4
Washington outer coast	247	56.3
Puget Sound	8972	51.6
British Columbia	7671	66.4
Southeastern Alaska	2498	58.2
Kodiak Island and Alaska Peninsula	5129	68.0
Japan	476	100.0

[a] All data except that by Orcutt (1950) for California and that for British Columbia are from table 5 of Hubbs and Kuronuma (1942).

However, use of a single figure to describe sinistrality for a large region may be misleading. Off British Columbia there is a general trend to a higher percentage of sinistral specimens proceeding from southernmost to northern areas (Fig. 1). Off the Fraser River estuary in the southern Strait of Georgia (about 49°N lat) samples of *P. stellatus* are about 51% sinistral, virtually identical to the percentage found in samples from Puget Sound immediately to the south. On the Two Peaks ground of northern Hecate Strait (54°20′N lat) samples are about 78% sinistral.

The most anomalous data in Fig. 1 are those from grounds off the west coast of Vancouver Island (about 49°N lat). However, there is more reason to consider the west coast samples in conjunction with offshore samples rather than with the inshore and Strait of Georgia samples. In this context the mean of 65.4% for the west coast lies between the 50–60% sinistral observed off the Oregon and Washington coasts, respectively, and the 76% for all of Hecate Strait.

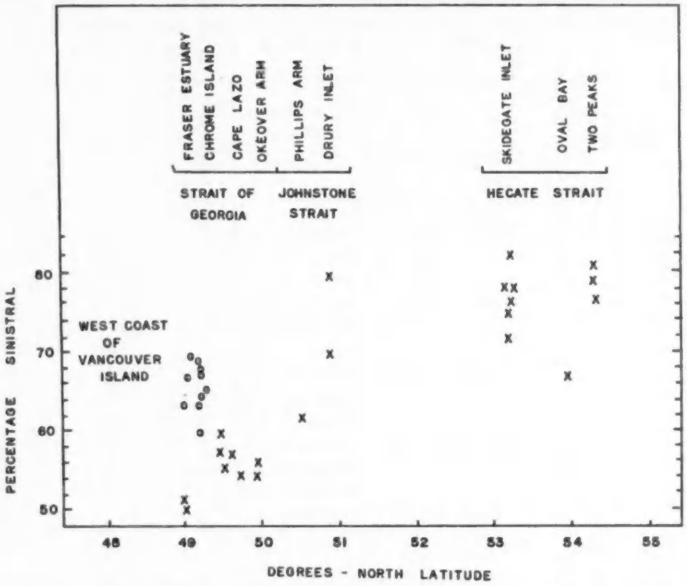

FIG. 1. Percentage sinistrality in *Platichthys stellatus* among samples collected off British Columbia. Data from west coast of Vancouver Island are shown by open circles.

The British Columbia samples were examined for: (1) the between-year variation within an area of percentage sinistral by comparable length intervals; (2) the between-area variation in percentage sinistral by comparable length intervals (percentages were transformed to angles prior to calculation); (3) within-year variation in an area of percentage sinistral by size groups.

Analysis of variance on seven sets of within-area data showed that between-year variations in an area were greater than between-length variations (Table 2) but neither between-year nor between-length variations were significant ($P < 0.05$). All four tests of between-area variations showed significant differences between areas. There were differences between samples taken off the west coast of Vancouver Island and Hecate Strait, between those from the west coast of Vancouver Island and the Fraser River estuary, between those from Hecate Strait and the Fraser River estuary, and between those from Cape Lazo and the Fraser River estuary. Chi-square tests on the numbers sinistral and dextral by length-groups within samples showed no apparent trend within samples and total chi-squares were not significant ($P < 0.05$; Table 4). This suggests no differential viability between dextral and sinistral forms of *P. stellatus*.

For the tests to be completely meaningful, the length groups compared between areas should represent the same year-classes. If growth differences exist between areas it might weaken the comparison. Further, comparisons of

TABLE 2. Results of analysis of variance tests for between-year and between-area variations in percentage sinistral in *Platichthys stellatus* off British Columbia.

Area	Years	Source of variance	df	F ratio
		Between years		
Lower west coast of Vancouver Is.	1955, 1959-61	Between years	3	1.06
		Between lengths	4	0.31
		Error (Y × L)	12	
	1962, 1964	Between years	1	1.51
		Between lengths	3	0.21
		Error (Y × L)	3	
Fraser R.	1957, 1962	Between years	1	3.75
		Between lengths	3	0.32
		Error (Y × L)	3	
Cape Lazo	1955, 1958, 1961	Between years	2	1.93
		Between lengths	3	0.25
		Error (Y × L)	6	
	1962, 1964	Between years	1	0.55
		Between lengths	2	0.09
		Error (Y × L)	2	
Upper Hecate Strait	1946, 1947	Between years	1	0.26
		Between lengths	3	0.32
		Error (Y × L)	3	
	1963, 1964	Between years	1	1.35
		Between lengths	4	0.96
		Error (Y × L)	4	
		Between areas		
Lower west coast of Vancouver Is. and Hecate Strait		Between areas	1	11.27
		Between lengths	7	0.55
		Error (A × L)	7	
Lower west coast of Vancouver Is. and Fraser R. estuary		Between areas	1	15.33
		Between lengths	4	2.35
		Error (A × L)	4	
Hecate Strait and Fraser R. estuary		Between areas	1	31.49
		Between lengths	4	2.36
		Error (A × L)	4	
Cape Lazo and Fraser R. estuary		Between areas	1	9.01
		Between lengths	4	4.91
		Error (A × L)	4	

sinistrality within an area to determine differences in viability should be made on fish of the same year-class or classes in successive time intervals. The sampling was not intensive enough to permit such a study. Although the conclusions are tentative, they do suggest the presence of a number of discrete local stocks of *P. stellatus*.

TABLE 3. Percentage sinistral by size groups in *Platichthys stellatus* off British Columbia. Number in a size group was 20 or greater.

Midpoint of length	Lower west coast Vancouver Is.						Johnstone Strait			Fraser R.		Cape Lazo					Upper Hecate Strait					
interval (*cm*)	1955	1959	1960	1961	1962	1964	1960	1961	1964	1957	1962	1955	1958	1961	1962	1964	1946	1947	1961	1962	1963	1964
32																39						
35								71			49	62	73	50		55						
38			66				76	71	74	49	47	48	71	58		55						
41			60		62	61	66	63	51	55	43	44	63	60	64	52				61	68	
44	54	78	65	68	71	64	64	61	62	49	49	54	52	65	46	67	75	89		66	88	
47	53	62	67	64	63	66	78	57	57	55	48	73			55	68	81	73	85	61	83	78
50	65	64	68	61	74	52	67	62			68	71			62		74	79	75	71	73	79
53	68	75	59	65	64		73	55									79	77	80	72	79	76
56	76	70	63	52			67	56										72	76	89	75	96
59		59	64																77		67	76
62		65																	82		81	
65																			82		87	
68																			73			
No. of fish	117	273	414	176	169	125	285	645	155	177	262	209	144	199	134	350	215	249	679	374	529	166

TABLE 4. Results of chi-square tests of numbers sinistral and dextral in various length groups of *Platichthys stellatus*.

Area	Year	No. of length intervals[a]	No. of fish	Chi-square	df
Lower west coast of Vancouver Is.	1955	5	117	3.79	4
	1959	7	273	5.24	6
	1960	8	414	2.01	7
	1961	5	176	1.70	4
	1962	5	169	1.89	4
	1964	4	125	1.03	3
Johnstone Strait	1960	7	285	4.03	6
	1961	8	645	7.65	7
	1964	4	155	4.13	3
Fraser R.	1957	4	177	0.69	3
	1962	6	262	4.23	5
Cape Lazo	1955	6	209	9.90	5
	1958	4	144	4.20	3
	1961	4	199	2.21	3
	1962	4	134	2.46	3
	1964	6	350	6.97	5
Upper Hecate Strait	1946	4	215	1.08	3
	1947	5	249	3.09	4
	1961	8	679	3.74	7
	1962	6	374	9.47	5
	1963	9	529	12.11	8
	1964	5	166	4.45	4

[a] See Table 3.

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